

HUMAN GENES AND GENE EXPRESSION PRODUCTS XVI

Field of the Invention

5 The present invention relates to polynucleotides of human origin and the encoded gene products.

Background of the Invention

Identification of novel polynucleotides, particularly those that encode an expressed gene product, is important in the advancement of drug discovery, diagnostic technologies, and the understanding of the progression and nature of complex diseases such as cancer. Identification of genes expressed in different cell types isolated from sources that differ in disease state or stage, developmental stage, exposure to various environmental factors, the tissue of origin, the species from which the tissue was isolated, and the like is key to identifying the genetic factors that are responsible for the phenotypes associated with these various differences.

15 This invention provides novel human polynucleotides, the polypeptides encoded by these polynucleotides, and the genes and proteins corresponding to these novel polynucleotides.

Summary of the Invention

This invention relates to novel human polynucleotides and variants thereof, their encoded polypeptides and variants thereof, to genes corresponding to these polynucleotides and to proteins expressed by the genes. The invention also relates to diagnostics and therapeutics comprising such novel human polynucleotides, their corresponding genes or gene products, including probes, antisense nucleotides, and antibodies. The polynucleotides of the invention correspond to a polynucleotide comprising the sequence information of at least one of SEQ ID NOS:1-316.

25 Various aspects and embodiments of the invention will be readily apparent to the ordinarily skilled artisan upon reading the description provided herein.

Brief Description of the Figures

Figures 1A-1B is a comparison of SEQ ID NO:315 and clone H72034 (SEQ ID NO:317).
30 Figure 2 is a comparison of SEQ ID NO:316 and clone AA707002 (SEQ ID NO:318).

Detailed Description of the Invention

The invention relates to polynucleotides comprising the disclosed nucleotide sequences, to full length cDNA, mRNA genomic sequences, and genes corresponding to these sequences and degenerate variants thereof, and to polypeptides encoded by the polynucleotides of the invention and polypeptide variants. The following detailed description describes the polynucleotide compositions encompassed by the invention, methods for obtaining cDNA or genomic DNA

encoding a full-length gene product, expression of these polynucleotides and genes, identification of structural motifs of the polynucleotides and genes, identification of the function of a gene product encoded by a gene corresponding to a polynucleotide of the invention, use of the provided polynucleotides as probes and in mapping and in tissue profiling, use of the corresponding polypeptides and other gene products to raise antibodies, and use of the polynucleotides and their encoded gene products for therapeutic and diagnostic purposes.

Polynucleotide Compositions

The scope of the invention with respect to polynucleotide compositions includes, but is not necessarily limited to, polynucleotides having a sequence set forth in any one of SEQ ID NOS:1-316; polynucleotides obtained from the biological materials described herein or other biological sources (particularly human sources) by hybridization under stringent conditions (particularly conditions of high stringency); genes corresponding to the provided polynucleotides; variants of the provided polynucleotides and their corresponding genes, particularly those variants that retain a biological activity of the encoded gene product (*e.g.*, a biological activity ascribed to a gene product corresponding to the provided polynucleotides as a result of the assignment of the gene product to a protein family(ies) and/or identification of a functional domain present in the gene product). Other nucleic acid compositions contemplated by and within the scope of the present invention will be readily apparent to one of ordinary skill in the art when provided with the disclosure here.

"Polynucleotide" and "nucleic acid" as used herein with reference to nucleic acids of the composition is not intended to be limiting as to the length or structure of the nucleic acid unless specifically indicated.

The invention features polynucleotides that are expressed in human tissue, specifically human colon, breast, and/or lung tissue. Novel nucleic acid compositions of the invention of particular interest comprise a sequence set forth in any one of SEQ ID NOS:1-316 or an identifying sequence thereof. An "identifying sequence" is a contiguous sequence of residues at least about 10 nt to about 20 nt in length, usually at least about 50 nt to about 100 nt in length, that uniquely identifies a polynucleotide sequence, *e.g.*, exhibits less than 90%, usually less than about 80% to about 85% sequence identity to any contiguous nucleotide sequence of more than about 20 nt. Thus, the subject novel nucleic acid compositions include full length cDNAs or mRNAs that encompass an identifying sequence of contiguous nucleotides from any one of SEQ ID NOS: 1-316.

The polynucleotides of the invention also include polynucleotides having sequence similarity or sequence identity. Nucleic acids having sequence similarity are detected by hybridization under low stringency conditions, for example, at 50°C and 10XSSC (0.9 M saline/0.09 M sodium citrate) and remain bound when subjected to washing at 55°C in 1XSSC.

Sequence identity can be determined by hybridization under stringent conditions, for example, at 50°C or higher and 0.1XSSC (9 mM saline/0.9 mM sodium citrate). Hybridization methods and conditions are well known in the art, see, *e.g.*, USPN 5,707,829. Nucleic acids that are substantially identical to the provided polynucleotide sequences, *e.g.* allelic variants, genetically altered versions of the gene, *etc.*, bind to the provided polynucleotide sequences (SEQ ID NOS:1-316) under stringent hybridization conditions. By using probes, particularly labeled probes of DNA sequences, one can isolate homologous or related genes. The source of homologous genes can be any species, *e.g.* primate species, particularly human; rodents, such as rats and mice; canines, felines, bovines, ovines, equines, yeast, nematodes, *etc.*

Preferably, hybridization is performed using at least 15 contiguous nucleotides (nt) of at least one of SEQ ID NOS:1-316. That is, when at least 15 contiguous nt of one of the disclosed SEQ ID NOS. is used as a probe, the probe will preferentially hybridize with a nucleic acid comprising the complementary sequence, allowing the identification and retrieval of the nucleic acids that uniquely hybridize to the selected probe. Probes from more than one SEQ ID NO. can hybridize with the same nucleic acid if the cDNA from which they were derived corresponds to one mRNA. Probes of more than 15 nt can be used, *e.g.*, probes of from about 18 nt to about 100 nt, but 15 nt represents sufficient sequence for unique identification.

The polynucleotides of the invention also include naturally occurring variants of the nucleotide sequences (*e.g.*, degenerate variants, allelic variants, *etc.*). Variants of the polynucleotides of the invention are identified by hybridization of putative variants with nucleotide sequences disclosed herein, preferably by hybridization under stringent conditions. For example, by using appropriate wash conditions, variants of the polynucleotides of the invention can be identified where the allelic variant exhibits at most about 25-30% base pair (bp) mismatches relative to the selected polynucleotide probe. In general, allelic variants contain 15-25% bp mismatches, and can contain as little as even 5-15%, or 2-5%, or 1-2% bp mismatches, as well as a single bp mismatch.

The invention also encompasses homologs corresponding to the polynucleotides of SEQ ID NOS:1-316, where the source of homologous genes can be any mammalian species, *e.g.*, primate species, particularly human; rodents, such as rats; canines, felines, bovines, ovines, equines, yeast, nematodes, *etc.* Between mammalian species, *e.g.*, human and mouse, homologs generally have substantial sequence similarity, *e.g.*, at least 75% sequence identity, usually at least 90%, more usually at least 95% between nucleotide sequences. Sequence similarity is calculated based on a reference sequence, which may be a subset of a larger sequence, such as a conserved motif, coding region, flanking region, *etc.* A reference sequence will usually be at least about 18 contiguous nt long, more usually at least about 30 nt long, and may extend to the complete

sequence that is being compared. Algorithms for sequence analysis are known in the art, such as gapped BLAST, described in Altschul, et al. *Nucleic Acids Res.* (1997) 25:3389-3402.

In general, variants of the invention have a sequence identity greater than at least about 65%, preferably at least about 75%, more preferably at least about 85%, and can be greater than at least about 90% or more as determined by the Smith-Waterman homology search algorithm as implemented in MPSRCH program (Oxford Molecular). For the purposes of this invention, a preferred method of calculating percent identity is the Smith-Waterman algorithm, using the following. Global DNA sequence identity must be greater than 65% as determined by the Smith-Waterman homology search algorithm as implemented in MPSRCH program (Oxford Molecular) using an affine gap search with the following search parameters: gap open penalty, 12; and gap extension penalty, 1.

The subject nucleic acids can be cDNAs or genomic DNAs, as well as fragments thereof, particularly fragments that encode a biologically active gene product and/or are useful in the methods disclosed herein (e.g., in diagnosis, as a unique identifier of a differentially expressed gene of interest, etc.). The term "cDNA" as used herein is intended to include all nucleic acids that share the arrangement of sequence elements found in native mature mRNA species, where sequence elements are exons and 3' and 5' non-coding regions. Normally mRNA species have contiguous exons, with the intervening introns, when present, being removed by nuclear RNA splicing, to create a continuous open reading frame encoding a polypeptide of the invention.

A genomic sequence of interest comprises the nucleic acid present between the initiation codon and the stop codon, as defined in the listed sequences, including all of the introns that are normally present in a native chromosome. It can further include the 3' and 5' untranslated regions found in the mature mRNA. It can further include specific transcriptional and translational regulatory sequences, such as promoters, enhancers, etc., including about 1 kb, but possibly more, of flanking genomic DNA at either the 5' and 3' end of the transcribed region. The genomic DNA can be isolated as a fragment of 100 kbp or smaller; and substantially free of flanking chromosomal sequence. The genomic DNA flanking the coding region, either 3' and 5', or internal regulatory sequences as sometimes found in introns, contains sequences required for proper tissue, stage-specific, or disease-state specific expression.

The nucleic acid compositions of the subject invention can encode all or a part of the subject polypeptides. Double or single stranded fragments can be obtained from the DNA sequence by chemically synthesizing oligonucleotides in accordance with conventional methods, by restriction enzyme digestion, by PCR amplification, etc. Isolated polynucleotides and polynucleotide fragments of the invention comprise at least about 10, about 15, about 20, about 35, about 50, about 100, about 150 to about 200, about 250 to about 300, or about 350 contiguous nt selected from the polynucleotide sequences as shown in SEQ ID NOS:1-316. For the most part,

fragments will be of at least 15 nt, usually at least 18 nt or 25 nt, and up to at least about 50 contiguous nt in length or more. In a preferred embodiment, the polynucleotide molecules comprise a contiguous sequence of at least 12 nt selected from the group consisting of the polynucleotides shown in SEQ ID NOS:1-316.

5 Probes specific to the polynucleotides of the invention can be generated using the polynucleotide sequences disclosed in SEQ ID NOS:1-316. The probes are preferably at least about a 12, 15, 16, 18, 20, 22, 24, or 25 nt fragment of a corresponding contiguous sequence of SEQ ID NOS:1-316, and can be less than 2, 1, 0.5, 0.1, or 0.05 kb in length. The probes can be synthesized chemically or can be generated from longer polynucleotides using restriction enzymes.
10 The probes can be labeled, for example, with a radioactive, biotinylated, or fluorescent tag. Preferably, probes are designed based upon an identifying sequence of a polynucleotide of one of SEQ ID NOS:1-316. More preferably, probes are designed based on a contiguous sequence of one of the subject polynucleotides that remain unmasked following application of a masking program for masking low complexity (*e.g.*, XBLAST) to the sequence., *i.e.*, one would select an unmasked
15 region, as indicated by the polynucleotides outside the poly-n stretches of the masked sequence produced by the masking program.

The polynucleotides of the subject invention are isolated and obtained in substantial purity, generally as other than an intact chromosome. Usually, the polynucleotides, either as DNA or RNA, will be obtained substantially free of other naturally-occurring nucleic acid sequences,
20 generally being at least about 50%, usually at least about 90% pure and are typically "recombinant", *e.g.*, flanked by one or more nucleotides with which it is not normally associated on a naturally occurring chromosome.

The polynucleotides of the invention can be provided as a linear molecule or within a circular molecule, and can be provided within autonomously replicating molecules (vectors) or
25 within molecules without replication sequences. Expression of the polynucleotides can be regulated by their own or by other regulatory sequences known in the art. The polynucleotides of the invention can be introduced into suitable host cells using a variety of techniques available in the art, such as transferrin polycation-mediated DNA transfer, transfection with naked or encapsulated nucleic acids, liposome-mediated DNA transfer, intracellular transportation of DNA-coated latex
30 beads, protoplast fusion, viral infection, electroporation, gene gun, calcium phosphate-mediated transfection, and the like.

The subject nucleic acid compositions can be used to, for example, produce polypeptides, as probes for the detection of mRNA of the invention in biological samples (*e.g.*, extracts of human cells) to generate additional copies of the polynucleotides, to generate ribozymes or antisense
35 oligonucleotides, and as single stranded DNA probes or as triple-strand forming oligonucleotides. The probes described herein can be used to, for example, determine the presence or absence of the

polynucleotide sequences as shown in SEQ ID NOS:1-316 or variants thereof in a sample. These and other uses are described in more detail below.

Use of Polynucleotides to Obtain Full-Length cDNA, Gene, and Promoter Region

Full-length cDNA molecules comprising the disclosed polynucleotides are obtained as follows. A polynucleotide having a sequence of one of SEQ ID NOS:1-316, or a portion thereof comprising at least 12, 15, 18, or 20 nt, is used as a hybridization probe to detect hybridizing members of a cDNA library using probe design methods, cloning methods, and clone selection techniques such as those described in USPN 5,654,173. Libraries of cDNA are made from selected tissues, such as normal or tumor tissue, or from tissues of a mammal treated with, for example, a pharmaceutical agent. Preferably, the tissue is the same as the tissue from which the polynucleotides of the invention were isolated, as both the polynucleotides described herein and the cDNA represent expressed genes. Most preferably, the cDNA library is made from the biological material described herein in the Examples. The choice of cell type for library construction can be made after the identity of the protein encoded by the gene corresponding to the polynucleotide of the invention is known. This will indicate which tissue and cell types are likely to express the related gene, and thus represent a suitable source for the mRNA for generating the cDNA. Where the provided polynucleotides are isolated from cDNA libraries, the libraries are prepared from mRNA of human colon cells, more preferably, human colon cancer cells, even more preferably, from a highly metastatic colon cell, Kml2L4-A.

Techniques for producing and probing nucleic acid sequence libraries are described, for example, in Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 2nd Ed., (1989) Cold Spring Harbor Press, Cold Spring Harbor, NY. The cDNA can be prepared by using primers based on sequence from SEQ ID NOS:1-316. In one embodiment, the cDNA library can be made from only poly-adenylated mRNA. Thus, poly-T primers can be used to prepare cDNA from the mRNA.

Members of the library that are larger than the provided polynucleotides, and preferably that encompass the complete coding sequence of the native message, are obtained. In order to confirm that the entire cDNA has been obtained, RNA protection experiments are performed as follows. Hybridization of a full-length cDNA to an mRNA will protect the RNA from RNase degradation. If the cDNA is not full length, then the portions of the mRNA that are not hybridized will be subject to RNase degradation. This is assayed, as is known in the art, by changes in electrophoretic mobility on polyacrylamide gels, or by detection of released monoribonucleotides. Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 2nd Ed., (1989) Cold Spring Harbor Press, Cold Spring Harbor, NY. In order to obtain additional sequences 5' to the end of a partial cDNA, 5' RACE (*PCR Protocols: A Guide to Methods and Applications*, (1990) Academic Press, Inc.) can be performed.

Genomic DNA is isolated using the provided polynucleotides in a manner similar to the isolation of full-length cDNAs. Briefly, the provided polynucleotides, or portions thereof, are used as probes to libraries of genomic DNA. Preferably, the library is obtained from the cell type that was used to generate the polynucleotides of the invention, but this is not essential. Most preferably, the genomic DNA is obtained from the biological material described herein in the Examples. Such libraries can be in vectors suitable for carrying large segments of a genome, such as P1 or YAC, as described in detail in Sambrook *et al.*, 9.4-9.30. In addition, genomic sequences can be isolated from human BAC libraries, which are commercially available from Research Genetics, Inc., Huntsville, Alabama, USA, for example. In order to obtain additional 5' or 3' sequences, chromosome walking is performed, as described in Sambrook *et al.*, such that adjacent and overlapping fragments of genomic DNA are isolated. These are mapped and pieced together, as is known in the art, using restriction digestion enzymes and DNA ligase.

Using the polynucleotide sequences of the invention, corresponding full-length genes can be isolated using both classical and PCR methods to construct and probe cDNA libraries. Using either method, Northern blots, preferably, are performed on a number of cell types to determine which cell lines express the gene of interest at the highest level. Classical methods of constructing cDNA libraries are taught in Sambrook *et al.*, *supra*. With these methods, cDNA can be produced from mRNA and inserted into viral or expression vectors. Typically, libraries of mRNA comprising poly(A) tails can be produced with poly(T) primers. Similarly, cDNA libraries can be produced using the instant sequences as primers.

PCR methods are used to amplify the members of a cDNA library that comprise the desired insert. In this case, the desired insert will contain sequence from the full length cDNA that corresponds to the instant polynucleotides. Such PCR methods include gene trapping and RACE methods. Gene trapping entails inserting a member of a cDNA library into a vector. The vector then is denatured to produce single stranded molecules. Next, a substrate-bound probe, such a biotinylated oligo, is used to trap cDNA inserts of interest. Biotinylated probes can be linked to an avidin-bound solid substrate. PCR methods can be used to amplify the trapped cDNA. To trap sequences corresponding to the full length genes, the labeled probe sequence is based on the polynucleotide sequences of the invention. Random primers or primers specific to the library vector can be used to amplify the trapped cDNA. Such gene trapping techniques are described in Gruber *et al.*, WO 95/04745 and Gruber *et al.*, USPN 5,500,356. Kits are commercially available to perform gene trapping experiments from, for example, Life Technologies, Gaithersburg, Maryland, USA.

"Rapid amplification of cDNA ends," or RACE, is a PCR method of amplifying cDNAs from a number of different RNAs. The cDNAs are ligated to an oligonucleotide linker, and amplified by PCR using two primers. One primer is based on sequence from the instant

polynucleotides, for which full length sequence is desired, and a second primer comprises sequence that hybridizes to the oligonucleotide linker to amplify the cDNA. A description of this methods is reported in WO 97/19110. In preferred embodiments of RACE, a common primer is designed to anneal to an arbitrary adaptor sequence ligated to cDNA ends (Apte and Siebert, *Biotechniques* 5 (1993) 15:890-893; Edwards *et al.*, *Nuc. Acids Res.* (1991) 19:5227-5232). When a single gene-specific RACE primer is paired with the common primer, preferential amplification of sequences between the single gene specific primer and the common primer occurs. Commercial cDNA pools modified for use in RACE are available.

Another PCR-based method generates full-length cDNA library with anchored ends 10 without needing specific knowledge of the cDNA sequence. The method uses lock-docking primers (I-VI), where one primer, poly TV (I-III) locks over the polyA tail of eukaryotic mRNA producing first strand synthesis and a second primer, polyGH (IV-VI) locks onto the polyC tail added by terminal deoxynucleotidyl transferase (TdT)(see, e.g., WO 96/40998).

The promoter region of a gene generally is located 5' to the initiation site for RNA 15 polymerase II. Hundreds of promoter regions contain the "TATA" box, a sequence such as TATTA or TATAA, which is sensitive to mutations. The promoter region can be obtained by performing 5' RACE using a primer from the coding region of the gene. Alternatively, the cDNA can be used as a probe for the genomic sequence, and the region 5' to the coding region is identified by "walking up." If the gene is highly expressed or differentially expressed, the promoter from the 20 gene can be of use in a regulatory construct for a heterologous gene.

Once the full-length cDNA or gene is obtained, DNA encoding variants can be prepared by site-directed mutagenesis, described in detail in Sambrook *et al.*, 15.3-15.63. The choice of codon or nucleotide to be replaced can be based on disclosure herein on optional changes in amino acids to achieve altered protein structure and/or function.

25 As an alternative method to obtaining DNA or RNA from a biological material, nucleic acid comprising nucleotides having the sequence of one or more polynucleotides of the invention can be synthesized. Thus, the invention encompasses nucleic acid molecules ranging in length from 15 nt (corresponding to at least 15 contiguous nt of one of SEQ ID NOS:1-316) up to a maximum length suitable for one or more biological manipulations, including replication and 30 expression, of the nucleic acid molecule. The invention includes but is not limited to (a) nucleic acid having the size of a full gene, and comprising at least one of SEQ ID NOS:1-316; (b) the nucleic acid of (a) also comprising at least one additional gene, operably linked to permit expression of a fusion protein; (c) an expression vector comprising (a) or (b); (d) a plasmid comprising (a) or (b); and (e) a recombinant viral particle comprising (a) or (b). Once provided 35 with the polynucleotides disclosed herein, construction or preparation of (a) - (e) are well within the skill in the art.

The sequence of a nucleic acid comprising at least 15 contiguous nt of at least any one of SEQ ID NOS:1-316, preferably the entire sequence of at least any one of SEQ ID NOS:1-316, is not limited and can be any sequence of A, T, G, and/or C (for DNA) and A, U, G, and/or C (for RNA) or modified bases thereof, including inosine and pseudouridine. The choice of sequence will depend on the desired function and can be dictated by coding regions desired, the intron-like regions desired, and the regulatory regions desired. Where the entire sequence of any one of SEQ ID NOS:1-316 is within the nucleic acid, the nucleic acid obtained is referred to herein as a polynucleotide comprising the sequence of any one of SEQ ID NOS:1-316.

Expression of Polypeptide Encoded by Full-Length cDNA or Full-Length Gene

The provided polynucleotides (e.g., a polynucleotide having a sequence of one of SEQ ID NOS:1-316), the corresponding cDNA, or the full-length gene is used to express a partial or complete gene product. Constructs of polynucleotides having sequences of SEQ ID NOS:1-316 can also be generated synthetically. Alternatively, single-step assembly of a gene and entire plasmid from large numbers of oligodeoxyribonucleotides is described by, e.g., Stemmer *et al.*, *Gene (Amsterdam)* (1995) 164(1):49-53. In this method, assembly PCR (the synthesis of long DNA sequences from large numbers of oligodeoxyribonucleotides (oligos)) is described. The method is derived from DNA shuffling (Stemmer, *Nature* (1994) 370:389-391), and does not rely on DNA ligase, but instead relies on DNA polymerase to build increasingly longer DNA fragments during the assembly process.

Appropriate polynucleotide constructs are purified using standard recombinant DNA techniques as described in, for example, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual, 2nd Ed.*, (1989) Cold Spring Harbor Press, Cold Spring Harbor, NY, and under current regulations described in United States Dept. of HHS, National Institute of Health (NIH) Guidelines for Recombinant DNA Research. The gene product encoded by a polynucleotide of the invention is expressed in any expression system, including, for example, bacterial, yeast, insect, amphibian and mammalian systems. Vectors, host cells and methods for obtaining expression in same are well known in the art. Suitable vectors and host cells are described in USPN 5,654,173.

Polynucleotide molecules comprising a polynucleotide sequence provided herein are generally propagated by placing the molecule in a vector. Viral and non-viral vectors are used, including plasmids. The choice of plasmid will depend on the type of cell in which propagation is desired and the purpose of propagation. Certain vectors are useful for amplifying and making large amounts of the desired DNA sequence. Other vectors are suitable for expression in cells in culture. Still other vectors are suitable for transfer and expression in cells in a whole animal or person. The choice of appropriate vector is well within the skill of the art. Many such vectors are available commercially. Methods for preparation of vectors comprising a desired sequence are well known in the art.

The polynucleotides set forth in SEQ ID NOS:1-316 or their corresponding full-length polynucleotides are linked to regulatory sequences as appropriate to obtain the desired expression properties. These can include promoters (attached either at the 5' end of the sense strand or at the 3' end of the antisense strand), enhancers, terminators, operators, repressors, and inducers. The promoters can be regulated or constitutive. In some situations it may be desirable to use conditionally active promoters, such as tissue-specific or developmental stage-specific promoters. These are linked to the desired nucleotide sequence using the techniques described above for linkage to vectors. Any techniques known in the art can be used.

When any of the above host cells, or other appropriate host cells or organisms, are used to replicate and/or express the polynucleotides or nucleic acids of the invention, the resulting replicated nucleic acid, RNA, expressed protein or polypeptide, is within the scope of the invention as a product of the host cell or organism. The product is recovered by any appropriate means known in the art.

Once the gene corresponding to a selected polynucleotide is identified, its expression can be regulated in the cell to which the gene is native. For example, an endogenous gene of a cell can be regulated by an exogenous regulatory sequence as disclosed in USPN 5,641,670.

Identification of Functional and Structural Motifs of Novel Genes Screening Against Publicly Available Databases

Translations of the nucleotide sequence of the provided polynucleotides, cDNAs or full genes can be aligned with individual known sequences. Similarity with individual sequences can be used to determine the activity of the polypeptides encoded by the polynucleotides of the invention. Also, sequences exhibiting similarity with more than one individual sequence can exhibit activities that are characteristic of either or both individual sequences.

The full length sequences and fragments of the polynucleotide sequences of the nearest neighbors can be used as probes and primers to identify and isolate the full length sequence corresponding to provided polynucleotides. The nearest neighbors can indicate a tissue or cell type to be used to construct a library for the full-length sequences corresponding to the provided polynucleotides.

Typically, a selected polynucleotide is translated in all six frames to determine the best alignment with the individual sequences. The sequences disclosed herein in the Sequence Listing are in a 5' to 3' orientation and translation in three frames can be sufficient (with a few specific exceptions as described in the Examples). These amino acid sequences are referred to, generally, as query sequences, which will be aligned with the individual sequences. Databases with individual sequences are described in "Computer Methods for Macromolecular Sequence Analysis" *Methods in Enzymology* (1996) 266, Doolittle, Academic Press, Inc., a division of Harcourt Brace & Co.,

San Diego, California, USA. Databases include GenBank, EMBL, and DNA Database of Japan (DDBJ).

Query and individual sequences can be aligned using the methods and computer programs described above, and include BLAST 2.0, available over the world wide web at a site supported by the National Center for Biotechnology Information, which is supported by the National Library of Medicine and the National Institutes of Health. See also Altschul, et al. *Nucleic Acids Res.* (1997) 25:3389-3402. Another alignment algorithm is Fasta, available in the Genetics Computing Group (GCG) package, Madison, Wisconsin, USA, a wholly owned subsidiary of Oxford Molecular Group, Inc. Other techniques for alignment are described in Doolittle, *supra*. Preferably, an alignment program that permits gaps in the sequence is utilized to align the sequences. The Smith-Waterman is one type of algorithm that permits gaps in sequence alignments. See *Meth. Mol. Biol.* (1997) 70: 173-187. Also, the GAP program using the Needleman and Wunsch alignment method can be utilized to align sequences. An alternative search strategy uses MPSRCH software, which runs on a MASPAR computer. MPSRCH uses a Smith-Waterman algorithm to score sequences on a massively parallel computer. This approach improves ability to identify sequences that are distantly related matches, and is especially tolerant of small gaps and nucleotide sequence errors. Amino acid sequences encoded by the provided polynucleotides can be used to search both protein and DNA databases. Incorporated herein by reference are all sequences that have been made public as of the filing date of this application by any of the DNA or protein sequence databases, including the patent databases (*e.g.*, GeneSeq). Also incorporated by reference are those sequences that have been submitted to these databases as of the filing date of the present application but not made public until after the filing date of the present application.

Results of individual and query sequence alignments can be divided into three categories: high similarity, weak similarity, and no similarity. Individual alignment results ranging from high similarity to weak similarity provide a basis for determining polypeptide activity and/or structure. Parameters for categorizing individual results include: percentage of the alignment region length where the strongest alignment is found, percent sequence identity, and p value. The percentage of the alignment region length is calculated by counting the number of residues of the individual sequence found in the region of strongest alignment, *e.g.*, contiguous region of the individual sequence that contains the greatest number of residues that are identical to the residues of the corresponding region of the aligned query sequence. This number is divided by the total residue length of the query sequence to calculate a percentage. For example, a query sequence of 20 amino acid residues might be aligned with a 20 amino acid region of an individual sequence. The individual sequence might be identical to amino acid residues 5, 9-15, and 17-19 of the query sequence. The region of strongest alignment is thus the region stretching from residue 9-19, an 11

amino acid stretch. The percentage of the alignment region length is: 11 (length of the region of strongest alignment) divided by (query sequence length) 20 or 55%.

Percent sequence identity is calculated by counting the number of amino acid matches between the query and individual sequence and dividing total number of matches by the number of residues of the individual sequences found in the region of strongest alignment. Thus, the percent identity in the example above would be 10 matches divided by 11 amino acids, or approximately, 90.9%

P value is the probability that the alignment was produced by chance. For a single alignment, the p value can be calculated according to Karlin *et al.*, *Proc. Natl. Acad. Sci.* (1990) 87:2264 and Karlin *et al.*, *Proc. Natl. Acad. Sci.* (1993) 90. The p value of multiple alignments using the same query sequence can be calculated using an heuristic approach described in Altschul *et al.*, *Nat. Genet.* (1994) 6:119. Alignment programs such as BLAST program can calculate the p value. See also Altschul *et al.*, *Nucleic Acids Res.* (1997) 25:3389-3402.

Another factor to consider for determining identity or similarity is the location of the similarity or identity. Strong local alignment can indicate similarity even if the length of alignment is short. Sequence identity scattered throughout the length of the query sequence also can indicate a similarity between the query and profile sequences. The boundaries of the region where the sequences align can be determined according to Doolittle, *supra*; BLAST 2.0 (see, *e.g.*, Altschul, *et al.* *Nucleic Acids Res.* (1997) 25:3389-3402) or FAST programs; or by determining the area where sequence identity is highest.

High Similarity. In general, in alignment results considered to be of high similarity, the percent of the alignment region length is typically at least about 55% of total length query sequence; more typically, at least about 58%; even more typically; at least about 60% of the total residue length of the query sequence. Usually, percent length of the alignment region can be as much as about 62%; more usually, as much as about 64%; even more usually, as much as about 66%. Further, for high similarity, the region of alignment, typically, exhibits at least about 75% of sequence identity; more typically, at least about 78%; even more typically; at least about 80% sequence identity. Usually, percent sequence identity can be as much as about 82%; more usually, as much as about 84%; even more usually, as much as about 86%.

The p value is used in conjunction with these methods. If high similarity is found, the query sequence is considered to have high similarity with a profile sequence when the p value is less than or equal to about 10^{-2} ; more usually; less than or equal to about 10^{-3} ; even more usually; less than or equal to about 10^{-4} . More typically, the p value is no more than about 10^{-5} ; more typically; no more than or equal to about 10^{-10} ; even more typically; no more than or equal to about 10^{-15} for the query sequence to be considered high similarity.

Weak Similarity. In general, where alignment results considered to be of weak similarity, there is no minimum percent length of the alignment region nor minimum length of alignment. A better showing of weak similarity is considered when the region of alignment is, typically, at least about 15 amino acid residues in length; more typically, at least about 20; even more typically, at least about 25 amino acid residues in length. Usually, length of the alignment region can be as much as about 30 amino acid residues; more usually, as much as about 40; even more usually, as much as about 60 amino acid residues. Further, for weak similarity, the region of alignment, typically, exhibits at least about 35% of sequence identity; more typically, at least about 40%; even more typically, at least about 45% sequence identity. Usually, percent sequence identity can be as much as about 50%; more usually, as much as about 55%; even more usually, as much as about 60%.

If low similarity is found, the query sequence is considered to have weak similarity with a profile sequence when the p value is usually less than or equal to about 10^{-2} ; more usually, less than or equal to about 10^{-3} ; even more usually, less than or equal to about 10^{-4} . More typically, the p value is no more than about 10^{-5} ; more usually, no more than or equal to about 10^{-10} ; even more usually, no more than or equal to about 10^{-15} for the query sequence to be considered weak similarity.

Similarity Determined by Sequence Identity Alone. Sequence identity alone can be used to determine similarity of a query sequence to an individual sequence and can indicate the activity of the sequence. Such an alignment, preferably, permits gaps to align sequences. Typically, the query sequence is related to the profile sequence if the sequence identity over the entire query sequence is at least about 15%; more typically, at least about 20%; even more typically, at least about 25%; even more typically, at least about 50%. Sequence identity alone as a measure of similarity is most useful when the query sequence is usually, at least 80 residues in length; more usually, 90 residues; even more usually, at least 95 amino acid residues in length. More typically, similarity can be concluded based on sequence identity alone when the query sequence is preferably 100 residues in length; more preferably, 120 residues in length; even more preferably, 150 amino acid residues in length.

Alignments with Profile and Multiple Aligned Sequences. Translations of the provided polynucleotides can be aligned with amino acid profiles that define either protein families or common motifs. Also, translations of the provided polynucleotides can be aligned to multiple sequence alignments (MSA) comprising the polypeptide sequences of members of protein families or motifs. Similarity or identity with profile sequences or MSAs can be used to determine the activity of the gene products (*e.g.*, polypeptides) encoded by the provided polynucleotides or corresponding cDNA or genes. For example, sequences that show an identity or similarity with a chemokine profile or MSA can exhibit chemokine activities.

Profiles can be designed manually by (1) creating an MSA, which is an alignment of the amino acid sequence of members that belong to the family and (2) constructing a statistical representation of the alignment. Such methods are described, for example, in Birney *et al.*, *Nucl. Acid Res.* (1996) 24(14): 2730-2739. MSAs of some protein families and motifs are publicly available. For example, the Genome Sequencing Center at the Washington University School of Medicine provides a web set (Pfam) which includes MSAs of 547 different families and motifs. These MSAs are described also in Sonnhammer *et al.*, *Proteins* (1997) 28: 405-420. Other sources over the world wide web include the site supported by the European Molecular Biology Laboratories in Heidelberg, Germany. A brief description of these MSAs is reported in Pascarella *et al.*, *Prot. Eng.* (1996) 9(3):249-251. Techniques for building profiles from MSAs are described in Sonnhammer *et al.*, *supra*; Birney *et al.*, *supra*; and "Computer Methods for Macromolecular Sequence Analysis," *Methods in Enzymology* (1996) 266, Doolittle, Academic Press, Inc., San Diego, California, USA.

Similarity between a query sequence and a protein family or motif can be determined by (a) comparing the query sequence against the profile and/or (b) aligning the query sequence with the members of the family or motif. Typically, a program such as Searchwise is used to compare the query sequence to the statistical representation of the multiple alignment, also known as a profile (see Birney *et al.*, *supra*). Other techniques to compare the sequence and profile are described in Sonnhammer *et al.*, *supra* and Doolittle, *supra*.

Next, methods described by Feng *et al.*, *J. Mol. Evol.* (1987) 25:351 and Higgins *et al.*, *CABIOS* (1989) 5:151 can be used to align the query sequence with the members of a family or motif, also known as a MSA. Sequence alignments can be generated using any of a variety of software tools. Examples include PileUp, which creates a multiple sequence alignment, and is described in Feng *et al.*, *J. Mol. Evol.* (1987) 25:351. Another method, GAP, uses the alignment method of Needleman *et al.*, *J. Mol. Biol.* (1970) 48:443. GAP is best suited for global alignment of sequences. A third method, BestFit, functions by inserting gaps to maximize the number of matches using the local homology algorithm of Smith *et al.*, *Adv. Appl. Math.* (1981) 2:482. In general, the following factors are used to determine if a similarity between a query sequence and a profile or MSA exists: (1) number of conserved residues found in the query sequence, (2) percentage of conserved residues found in the query sequence, (3) number of frameshifts, and (4) spacing between conserved residues.

Some alignment programs that both translate and align sequences can make any number of frameshifts when translating the nucleotide sequence to produce the best alignment. The fewer frameshifts needed to produce an alignment, the stronger the similarity or identity between the query and profile or MSAs. For example, a weak similarity resulting from no frameshifts can be a better indication of activity or structure of a query sequence, than a strong similarity resulting from

two frameshifts. Preferably, three or fewer frameshifts are found in an alignment; more preferably two or fewer frameshifts; even more preferably, one or fewer frameshifts; even more preferably, no frameshifts are found in an alignment of query and profile or MSAs.

Conserved residues are those amino acids found at a particular position in all or some of the family or motif members. Alternatively, a position is considered conserved if only a certain class of amino acids is found in a particular position in all or some of the family members. For example, the N-terminal position can contain a positively charged amino acid, such as lysine, arginine, or histidine.

Typically, a residue of a polypeptide is conserved when a class of amino acids or a single amino acid is found at a particular position in at least about 40% of all class members; more typically, at least about 50%; even more typically, at least about 60% of the members. Usually, a residue is conserved when a class or single amino acid is found in at least about 70% of the members of a family or motif; more usually, at least about 80%; even more usually, at least about 90%; even more usually, at least about 95%.

A residue is considered conserved when three unrelated amino acids are found at a particular position in the some or all of the members; more usually, two unrelated amino acids. These residues are conserved when the unrelated amino acids are found at particular positions in at least about 40% of all class member; more typically, at least about 50%; even more typically, at least about 60% of the members. Usually, a residue is conserved when a class or single amino acid is found in at least about 70% of the members of a family or motif; more usually, at least about 80%; even more usually, at least about 90%; even more usually, at least about 95%.

A query sequence has similarity to a profile or MSA when the query sequence comprises at least about 25% of the conserved residues of the profile or MSA; more usually, at least about 30%; even more usually; at least about 40%. Typically, the query sequence has a stronger similarity to a profile sequence or MSA when the query sequence comprises at least about 45% of the conserved residues of the profile or MSA; more typically, at least about 50%; even more typically; at least about 55%.

Identification of Secreted & Membrane-Bound Polypeptides

Both secreted and membrane-bound polypeptides of the present invention are of particular interest. For example, levels of secreted polypeptides can be assayed in body fluids that are convenient, such as blood, plasma, serum, and other body fluids such as urine, prostatic fluid and semen. Membrane-bound polypeptides are useful for constructing vaccine antigens or inducing an immune response. Such antigens would comprise all or part of the extracellular region of the membrane-bound polypeptides. Because both secreted and membrane-bound polypeptides comprise a fragment of contiguous hydrophobic amino acids, hydrophobicity predicting algorithms can be used to identify such polypeptides.

A signal sequence is usually encoded by both secreted and membrane-bound polypeptide genes to direct a polypeptide to the surface of the cell. The signal sequence usually comprises a stretch of hydrophobic residues. Such signal sequences can fold into helical structures. Membrane-bound polypeptides typically comprise at least one transmembrane region that possesses a stretch of hydrophobic amino acids that can transverse the membrane. Some transmembrane regions also exhibit a helical structure. Hydrophobic fragments within a polypeptide can be identified by using computer algorithms. Such algorithms include Hopp & Woods, *Proc. Natl. Acad. Sci. USA* (1981) 78:3824-3828; Kyte & Doolittle, *J. Mol. Biol.* (1982) 157: 105-132; and RAOAR algorithm, Degli Esposti *et al.*, *Eur. J. Biochem.* (1990) 190: 207-219.

Another method of identifying secreted and membrane-bound polypeptides is to translate the polynucleotides of the invention in all six frames and determine if at least 8 contiguous hydrophobic amino acids are present. Those translated polypeptides with at least 8; more typically, 10; even more typically, 12 contiguous hydrophobic amino acids are considered to be either a putative secreted or membrane bound polypeptide. Hydrophobic amino acids include alanine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tryptophan, tyrosine, and valine.

Identification of the Function of an Expression Product of a Full-Length Gene

Ribozymes, antisense constructs, and dominant negative mutants can be used to determine function of the expression product of a gene corresponding to a polynucleotide provided herein.

These methods and compositions are particularly useful where the provided novel polynucleotide exhibits no significant or substantial homology to a sequence encoding a gene of known function. Antisense molecules and ribozymes can be constructed from synthetic polynucleotides. Typically, the phosphoramidite method of oligonucleotide synthesis is used. See Beaucage *et al.*, *Tet. Lett.* (1981) 22:1859 and USPN 4,668,777. Automated devices for synthesis are available to create oligonucleotides using this chemistry. Examples of such devices include Biosearch 8600, Models 392 and 394 by Applied Biosystems, a division of Perkin-Elmer Corp., Foster City, California, USA; and Expedite by Perceptive Biosystems, Framingham, Massachusetts, USA. Synthetic RNA, phosphate analog oligonucleotides, and chemically derivatized oligonucleotides can also be produced, and can be covalently attached to other molecules. RNA oligonucleotides can be synthesized, for example, using RNA phosphoramidites. This method can be performed on an automated synthesizer, such as Applied Biosystems, Models 392 and 394, Foster City, California, USA.

Phosphorothioate oligonucleotides can also be synthesized for antisense construction. A sulfurizing reagent, such as tetraethylthiuram disulfide (TETD) in acetonitrile can be used to convert the internucleotide cyanoethyl phosphite to the phosphorothioate triester within 15 minutes at room temperature. TETD replaces the iodine reagent, while all other reagents used for standard

phosphoramidite chemistry remain the same. Such a synthesis method can be automated using Models 392 and 394 by Applied Biosystems, for example.

Oligonucleotides of up to 200 nt can be synthesized, more typically, 100 nt, more typically 50 nt; even more typically 30 to 40 nt. These synthetic fragments can be annealed and ligated together to construct larger fragments. See, for example, Sambrook *et al.*, *supra*. Trans-cleaving catalytic RNAs (ribozymes) are RNA molecules possessing endoribonuclease activity. Ribozymes are specifically designed for a particular target, and the target message must contain a specific nucleotide sequence. They are engineered to cleave any RNA species site-specifically in the background of cellular RNA. The cleavage event renders the mRNA unstable and prevents protein expression. Importantly, ribozymes can be used to inhibit expression of a gene of unknown function for the purpose of determining its function in an in vitro or in vivo context, by detecting the phenotypic effect. One commonly used ribozyme motif is the hammerhead, for which the substrate sequence requirements are minimal. Design of the hammerhead ribozyme, as well as therapeutic uses of ribozymes, are disclosed in Usman *et al.*, *Current Opin. Struct. Biol.* (1996) 6:527. Methods for production of ribozymes, including hairpin structure ribozyme fragments, methods of increasing ribozyme specificity, and the like are known in the art.

The hybridizing region of the ribozyme can be modified or can be prepared as a branched structure as described in Horn and Urdea, *Nucleic Acids Res.* (1989) 17:6959. The basic structure of the ribozymes can also be chemically altered in ways familiar to those skilled in the art, and chemically synthesized ribozymes can be administered as synthetic oligonucleotide derivatives modified by monomeric units. In a therapeutic context, liposome mediated delivery of ribozymes improves cellular uptake, as described in Birikh *et al.*, *Eur. J. Biochem.* (1997) 245:1.

Antisense nucleic acids are designed to specifically bind to RNA, resulting in the formation of RNA-DNA or RNA-RNA hybrids, with an arrest of DNA replication, reverse transcription or messenger RNA translation. Antisense polynucleotides based on a selected polynucleotide sequence can interfere with expression of the corresponding gene. Antisense polynucleotides are typically generated within the cell by expression from antisense constructs that contain the antisense strand as the transcribed strand. Antisense polynucleotides based on the disclosed polynucleotides will bind and/or interfere with the translation of mRNA comprising a sequence complementary to the antisense polynucleotide. The expression products of control cells and cells treated with the antisense construct are compared to detect the protein product of the gene corresponding to the polynucleotide upon which the antisense construct is based. The protein is isolated and identified using routine biochemical methods.

Given the extensive background literature and clinical experience in antisense therapy, one skilled in the art can use selected polynucleotides of the invention as additional potential therapeutics. The choice of polynucleotide can be narrowed by first testing them for binding to

"hot spot" regions of the genome of cancerous cells. If a polynucleotide is identified as binding to a "hot spot", testing the polynucleotide as an antisense compound in the corresponding cancer cells is warranted.

As an alternative method for identifying function of the gene corresponding to a polynucleotide disclosed herein, dominant negative mutations are readily generated for corresponding proteins that are active as homomultimers. A mutant polypeptide will interact with wild-type polypeptides (made from the other allele) and form a non-functional multimer. Thus, a mutation is in a substrate-binding domain, a catalytic domain, or a cellular localization domain. Preferably, the mutant polypeptide will be overproduced. Point mutations are made that have such an effect. In addition, fusion of different polypeptides of various lengths to the terminus of a protein can yield dominant negative mutants. General strategies are available for making dominant negative mutants (see, *e.g.*, Herskowitz, *Nature* (1987) 329:219). Such techniques can be used to create loss of function mutations, which are useful for determining protein function.

Polypeptides and Variants Thereof

The polypeptides of the invention include those encoded by the disclosed polynucleotides, as well as nucleic acids that, by virtue of the degeneracy of the genetic code, are not identical in sequence to the disclosed polynucleotides. Thus, the invention includes within its scope a polypeptide encoded by a polynucleotide having the sequence of any one of SEQ ID NOS:1-316 or a variant thereof.

In general, the term "polypeptide" as used herein refers to both the full length polypeptide encoded by the recited polynucleotide, the polypeptide encoded by the gene represented by the recited polynucleotide, as well as portions or fragments thereof. "Polypeptides" also includes variants of the naturally occurring proteins, where such variants are homologous or substantially similar to the naturally occurring protein, and can be of an origin of the same or different species as the naturally occurring protein (*e.g.*, human, murine, or some other species that naturally expresses the recited polypeptide, usually a mammalian species). In general, variant polypeptides have a sequence that has at least about 80%, usually at least about 90%, and more usually at least about 98% sequence identity with a differentially expressed polypeptide of the invention, as measured by BLAST 2.0 using the parameters described above. The variant polypeptides can be naturally or non-naturally glycosylated, *i.e.*, the polypeptide has a glycosylation pattern that differs from the glycosylation pattern found in the corresponding naturally occurring protein.

The invention also encompasses homologs of the disclosed polypeptides (or fragments thereof) where the homologs are isolated from other species, *i.e.* other animal or plant species, where such homologs, usually mammalian species, *e.g.* rodents, such as mice, rats; domestic animals, *e.g.*, horse, cow, dog, cat; and humans. By "homolog" is meant a polypeptide having at least about 35%, usually at least about 40% and more usually at least about 60% amino acid

sequence identity to a particular differentially expressed protein as identified above, where sequence identity is determined using the BLAST 2.0 algorithm, with the parameters described *supra*.

In general, the polypeptides of the subject invention are provided in a non-naturally occurring environment, e.g. are separated from their naturally occurring environment. In certain embodiments, the subject protein is present in a composition that is enriched for the protein as compared to a control. As such, purified polypeptide is provided, where by purified is meant that the protein is present in a composition that is substantially free of non-differentially expressed polypeptides, where by substantially free is meant that less than 90%, usually less than 60% and more usually less than 50% of the composition is made up of non-differentially expressed polypeptides.

Also within the scope of the invention are variants; variants of polypeptides include mutants, fragments, and fusions. Mutants can include amino acid substitutions, additions or deletions. The amino acid substitutions can be conservative amino acid substitutions or substitutions to eliminate non-essential amino acids, such as to alter a glycosylation site, a phosphorylation site or an acetylation site, or to minimize misfolding by substitution or deletion of one or more cysteine residues that are not necessary for function. Conservative amino acid substitutions are those that preserve the general charge, hydrophobicity/ hydrophilicity, and/or steric bulk of the amino acid substituted. Variants can be designed so as to retain or have enhanced biological activity of a particular region of the protein (e.g., a functional domain and/or, where the polypeptide is a member of a protein family, a region associated with a consensus sequence). Selection of amino acid alterations for production of variants can be based upon the accessibility (interior vs. exterior) of the amino acid (see, e.g., Go *et al*, *Int. J. Peptide Protein Res.* (1980) 15:211), the thermostability of the variant polypeptide (see, e.g., Querol *et al.*, *Prot. Eng.* (1996) 9:265), desired glycosylation sites (see, e.g., Olsen and Thomsen, *J. Gen. Microbiol.* (1991) 137:579), desired disulfide bridges (see, e.g., Clarke *et al.*, *Biochemistry* (1993) 32:4322; and Wakarchuk *et al.*, *Protein Eng.* (1994) 7:1379), desired metal binding sites (see, e.g., Toma *et al.*, *Biochemistry* (1991) 30:97, and Haezebrouck *et al.*, *Protein Eng.* (1993) 6:643), and desired substitutions with in proline loops (see, e.g., Masul *et al.*, *Appl. Env. Microbiol.* (1994) 60:3579). Cysteine-depleted muteins can be produced as disclosed in USPN 4,959,314.

Variants also include fragments of the polypeptides disclosed herein, particularly biologically active fragments and/or fragments corresponding to functional domains. Fragments of interest will typically be at least about 10 aa to at least about 15 aa in length, usually at least about 50 aa in length, and can be as long as 300 aa in length or longer, but will usually not exceed about 1000 aa in length, where the fragment will have a stretch of amino acids that is identical to a polypeptide encoded by a polynucleotide having a sequence of any SEQ ID NOS:1-316, or a

homolog thereof. The protein variants described herein are encoded by polynucleotides that are within the scope of the invention. The genetic code can be used to select the appropriate codons to construct the corresponding variants.

Computer-Related Embodiments

5 In general, a library of polynucleotides is a collection of sequence information, which information is provided in either biochemical form (*e.g.*, as a collection of polynucleotide molecules), or in electronic form (*e.g.*, as a collection of polynucleotide sequences stored in a computer-readable form, as in a computer system and/or as part of a computer program). The sequence information of the polynucleotides can be used in a variety of ways, *e.g.*, as a resource for
10 gene discovery, as a representation of sequences expressed in a selected cell type (*e.g.*, cell type markers), and/or as markers of a given disease or disease state. In general, a disease marker is a representation of a gene product that is present in all cells affected by disease either at an increased or decreased level relative to a normal cell (*e.g.*, a cell of the same or similar type that is not substantially affected by disease). For example, a polynucleotide sequence in a library can be a
15 polynucleotide that represents an mRNA, polypeptide, or other gene product encoded by the polynucleotide, that is either overexpressed or underexpressed in a breast ductal cell affected by cancer relative to a normal (*i.e.*, substantially disease-free) breast cell.

The nucleotide sequence information of the library can be embodied in any suitable form, *e.g.*, electronic or biochemical forms. For example, a library of sequence information embodied in
20 electronic form comprises an accessible computer data file (or, in biochemical form, a collection of nucleic acid molecules) that contains the representative nucleotide sequences of genes that are differentially expressed (*e.g.*, overexpressed or underexpressed) as between, for example, i) a cancerous cell and a normal cell; ii) a cancerous cell and a dysplastic cell; iii) a cancerous cell and a cell affected by a disease or condition other than cancer; iv) a metastatic cancerous cell and a
25 normal cell and/or non-metastatic cancerous cell; v) a malignant cancerous cell and a non-malignant cancerous cell (or a normal cell) and/or vi) a dysplastic cell relative to a normal cell. Other combinations and comparisons of cells affected by various diseases or stages of disease will be readily apparent to the ordinarily skilled artisan. Biochemical embodiments of the library include a collection of nucleic acids that have the sequences of the genes in the library, where the
30 nucleic acids can correspond to the entire gene in the library or to a fragment thereof, as described in greater detail below.

The polynucleotide libraries of the subject invention generally comprise sequence information of a plurality of polynucleotide sequences, where at least one of the polynucleotides has a sequence of any of SEQ ID NOS:1-316. By plurality is meant at least 2, usually at least 3
35 and can include up to all of SEQ ID NOS:1-316. The length and number of polynucleotides in the

library will vary with the nature of the library, *e.g.*, if the library is an oligonucleotide array, a cDNA array, a computer database of the sequence information, etc.

Where the library is an electronic library, the nucleic acid sequence information can be present in a variety of media. "Media" refers to a manufacture, other than an isolated nucleic acid molecule, that contains the sequence information of the present invention. Such a manufacture provides the genome sequence or a subset thereof in a form that can be examined by means not directly applicable to the sequence as it exists in a nucleic acid. For example, the nucleotide sequence of the present invention, *e.g.* the nucleic acid sequences of any of the polynucleotides of SEQ ID NOS:1-316, can be recorded on computer readable media, *e.g.* any medium that can be read and accessed directly by a computer. Such media include, but are not limited to: magnetic storage media, such as a floppy disc, a hard disc storage medium, and a magnetic tape; optical storage media such as CD-ROM; electrical storage media such as RAM and ROM; and hybrids of these categories such as magnetic/optical storage media. One of skill in the art can readily appreciate how any of the presently known computer readable mediums can be used to create a manufacture comprising a recording of the present sequence information. "Recorded" refers to a process for storing information on computer readable medium, using any such methods as known in the art. Any convenient data storage structure can be chosen, based on the means used to access the stored information. A variety of data processor programs and formats can be used for storage, *e.g.* word processing text file, database format, *etc.* In addition to the sequence information, electronic versions of the libraries of the invention can be provided in conjunction or connection with other computer-readable information and/or other types of computer-readable files (*e.g.*, searchable files, executable files, *etc.*, including, but not limited to, for example, search program software, *etc.*).

By providing the nucleotide sequence in computer readable form, the information can be accessed for a variety of purposes. Computer software to access sequence information is publicly available. For example, the gapped BLAST (Altschul *et al. Nucleic Acids Res.* (1997) 25:3389-3402) and BLAZE (Brutlag *et al. Comp. Chem.* (1993) 17:203) search algorithms on a Sybase system can be used to identify open reading frames (ORFs) within the genome that contain homology to ORFs from other organisms.

As used herein, "a computer-based system" refers to the hardware means, software means, and data storage means used to analyze the nucleotide sequence information of the present invention. The minimum hardware of the computer-based systems of the present invention comprises a central processing unit (CPU), input means, output means, and data storage means. A skilled artisan can readily appreciate that any one of the currently available computer-based systems are suitable for use in the present invention. The data storage means can comprise any manufacture comprising a recording of the present sequence information as described above, or a memory access means that can access such a manufacture.

"Search means" refers to one or more programs implemented on the computer-based system, to compare a target sequence or target structural motif, or expression levels of a polynucleotide in a sample, with the stored sequence information. Search means can be used to identify fragments or regions of the genome that match a particular target sequence or target motif.

5 A variety of known algorithms are publicly known and commercially available, *e.g.* MacPattern (EMBL), BLASTN and BLASTX (NCBI). A "target sequence" can be any polynucleotide or amino acid sequence of six or more contiguous nucleotides or two or more amino acids, preferably from about 10 to 100 amino acids or from about 30 to 300 nt. A variety of comparing means can be used to accomplish comparison of sequence information from a sample (*e.g.*, to analyze target
10 sequences, target motifs, or relative expression levels) with the data storage means. A skilled artisan can readily recognize that any one of the publicly available homology search programs can be used as the search means for the computer based systems of the present invention to accomplish comparison of target sequences and motifs. Computer programs to analyze expression levels in a sample and in controls are also known in the art.

15 A "target structural motif," or "target motif," refers to any rationally selected sequence or combination of sequences in which the sequence(s) are chosen based on a three-dimensional configuration that is formed upon the folding of the target motif, or on consensus sequences of regulatory or active sites. There are a variety of target motifs known in the art. Protein target motifs include, but are not limited to, enzyme active sites and signal sequences. Nucleic acid target
20 motifs include, but are not limited to, hairpin structures, promoter sequences and other expression elements such as binding sites for transcription factors.

A variety of structural formats for the input and output means can be used to input and output the information in the computer-based systems of the present invention. One format for an output means ranks the relative expression levels of different polynucleotides. Such presentation
25 provides a skilled artisan with a ranking of relative expression levels to determine a gene expression profile.

As discussed above, the "library" of the invention also encompasses biochemical libraries of the polynucleotides of SEQ ID NOS:1-316, *e.g.*, collections of nucleic acids representing the provided polynucleotides. The biochemical libraries can take a variety of forms, *e.g.*, a solution of
30 cDNAs, a pattern of probe nucleic acids stably associated with a surface of a solid support (*i.e.*, an array) and the like. Of particular interest are nucleic acid arrays in which one or more of SEQ ID NOS:1-316 is represented on the array. By array is meant an article of manufacture that has at least a substrate with at least two distinct nucleic acid targets on one of its surfaces, where the number of distinct nucleic acids can be considerably higher, typically being at least 10 nt, usually at
35 least 20 nt and often at least 25 nt. A variety of different array formats have been developed and are known to those of skill in the art. The arrays of the subject invention find use in a variety of

applications, including gene expression analysis, drug screening, mutation analysis and the like, as disclosed in the above-listed exemplary patent documents.

In addition to the above nucleic acid libraries, analogous libraries of polypeptides are also provided, where the where the polypeptides of the library will represent at least a portion of the polypeptides encoded by SEQ ID NOS:1-316.

Utilities

Use of Polynucleotide Probes in Mapping, and in Tissue Profiling

Polynucleotide probes, generally comprising at least 12 contiguous nt of a polynucleotide as shown in the Sequence Listing, are used for a variety of purposes, such as chromosome mapping of the polynucleotide and detection of transcription levels. Additional disclosure about preferred regions of the disclosed polynucleotide sequences is found in the Examples. A probe that hybridizes specifically to a polynucleotide disclosed herein should provide a detection signal at least 5-, 10-, or 20-fold higher than the background hybridization provided with other unrelated sequences.

Detection of Expression Levels. Nucleotide probes are used to detect expression of a gene corresponding to the provided polynucleotide. In Northern blots, mRNA is separated electrophoretically and contacted with a probe. A probe is detected as hybridizing to an mRNA species of a particular size. The amount of hybridization is quantitated to determine relative amounts of expression, for example under a particular condition. Probes are used for in situ hybridization to cells to detect expression. Probes can also be used *in vivo* for diagnostic detection of hybridizing sequences. Probes are typically labeled with a radioactive isotope. Other types of detectable labels can be used such as chromophores, fluors, and enzymes. Other examples of nucleotide hybridization assays are described in WO92/02526 and USPN 5,124,246.

Alternatively, the Polymerase Chain Reaction (PCR) is another means for detecting small amounts of target nucleic acids (see, e.g., Mullis *et al.*, *Meth. Enzymol.* (1987) 155:335; USPN 4,683,195; and USPN 4,683,202). Two primer polynucleotides nucleotides that hybridize with the target nucleic acids are used to prime the reaction. The primers can be composed of sequence within or 3' and 5' to the polynucleotides of the Sequence Listing. Alternatively, if the primers are 3' and 5' to these polynucleotides, they need not hybridize to them or the complements. After amplification of the target with a thermostable polymerase, the amplified target nucleic acids can be detected by methods known in the art, e.g., Southern blot. mRNA or cDNA can also be detected by traditional blotting techniques (e.g., Southern blot, Northern blot, etc.) described in Sambrook *et al.*, "Molecular Cloning: A Laboratory Manual" (New York, Cold Spring Harbor Laboratory, 1989) (e.g., without PCR amplification). In general, mRNA or cDNA generated from mRNA using a polymerase enzyme can be purified and separated using gel electrophoresis, and transferred to a

solid support, such as nitrocellulose. The solid support is exposed to a labeled probe, washed to remove any unhybridized probe, and duplexes containing the labeled probe are detected.

Mapping. Polynucleotides of the present invention can be used to identify a chromosome on which the corresponding gene resides. Such mapping can be useful in identifying the function of the polynucleotide-related gene by its proximity to other genes with known function. Function can also be assigned to the polynucleotide-related gene when particular syndromes or diseases map to the same chromosome. For example, use of polynucleotide probes in identification and quantification of nucleic acid sequence aberrations is described in USPN 5,783,387. An exemplary mapping method is fluorescence in situ hybridization (FISH), which facilitates comparative genomic hybridization to allow total genome assessment of changes in relative copy number of DNA sequences (see, e.g., Valdes *et al.*, *Methods in Molecular Biology* (1997) 68:1). Polynucleotides can also be mapped to particular chromosomes using, for example, radiation hybrids or chromosome-specific hybrid panels. See Leach *et al.*, *Advances in Genetics*, (1995) 33:63-99; Walter *et al.*, *Nature Genetics* (1994) 7:22; Walter and Goodfellow, *Trends in Genetics* (1992) 9:352. Panels for radiation hybrid mapping are available from Research Genetics, Inc., Huntsville, Alabama, USA. Databases for markers using various panels are available via the world wide web at sites supported by the Stanford Human Genome Center (Stanford University) and the Whitehead Institute for Biomedical Research/MIT Center for Genome Research. The statistical program RHMAP can be used to construct a map based on the data from radiation hybridization with a measure of the relative likelihood of one order versus another. RHMAP is available via the world wide web at a site supported by the Center for Statistical Genetics at the University of Michigan School of Public Health. In addition, commercial programs are available for identifying regions of chromosomes commonly associated with disease, such as cancer.

Tissue Typing or Profiling. Expression of specific mRNA corresponding to the provided polynucleotides can vary in different cell types and can be tissue-specific. This variation of mRNA levels in different cell types can be exploited with nucleic acid probe assays to determine tissue types. For example, PCR, branched DNA probe assays, or blotting techniques utilizing nucleic acid probes substantially identical or complementary to polynucleotides listed in the Sequence Listing can determine the presence or absence of the corresponding cDNA or mRNA.

Tissue typing can be used to identify the developmental organ or tissue source of a metastatic lesion by identifying the expression of a particular marker of that organ or tissue. If a polynucleotide is expressed only in a specific tissue type, and a metastatic lesion is found to express that polynucleotide, then the developmental source of the lesion has been identified. Expression of a particular polynucleotide can be assayed by detection of either the corresponding mRNA or the protein product. As would be readily apparent to any forensic scientist, the sequences disclosed herein are useful in differentiating human tissue from non-human tissue. In particular, these

sequences are useful to differentiate human tissue from bird, reptile, and amphibian tissue, for example.

Use of Polymorphisms. A polynucleotide of the invention can be used in forensics, genetic analysis, mapping, and diagnostic applications where the corresponding region of a gene is polymorphic in the human population. Any means for detecting a polymorphism in a gene can be used, including, but not limited to electrophoresis of protein polymorphic variants, differential sensitivity to restriction enzyme cleavage, and hybridization to allele-specific probes.

Antibody Production

Expression products of a polynucleotide of the invention, as well as the corresponding mRNA, cDNA, or complete gene, can be prepared and used for raising antibodies for experimental, diagnostic, and therapeutic purposes. For polynucleotides to which a corresponding gene has not been assigned, this provides an additional method of identifying the corresponding gene. The polynucleotide or related cDNA is expressed as described above, and antibodies are prepared. These antibodies are specific to an epitope on the polypeptide encoded by the polynucleotide, and can precipitate or bind to the corresponding native protein in a cell or tissue preparation or in a cell-free extract of an in vitro expression system.

Methods for production of antibodies that specifically bind a selected antigen are well known in the art. Immunogens for raising antibodies can be prepared by mixing a polypeptide encoded by a polynucleotide of the invention with an adjuvant, and/or by making fusion proteins with larger immunogenic proteins. Polypeptides can also be covalently linked to other larger immunogenic proteins, such as keyhole limpet hemocyanin. Immunogens are typically administered intradermally, subcutaneously, or intramuscularly to experimental animals such as rabbits, sheep, and mice, to generate antibodies. Monoclonal antibodies can be generated by isolating spleen cells and fusing myeloma cells to form hybridomas. Alternatively, the selected polynucleotide is administered directly, such as by intramuscular injection, and expressed in vivo. The expressed protein generates a variety of protein-specific immune responses, including production of antibodies, comparable to administration of the protein.

Preparations of polyclonal and monoclonal antibodies specific for polypeptides encoded by a selected polynucleotide are made using standard methods known in the art. The antibodies specifically bind to epitopes present in the polypeptides encoded by polynucleotides disclosed in the Sequence Listing. Typically, at least 6, 8, 10, or 12 contiguous amino acids are required to form an epitope. Epitopes that involve non-contiguous amino acids may require a longer polypeptide, e.g., at least 15, 25, or 50 amino acids. Antibodies that specifically bind to human polypeptides encoded by the provided polypeptides should provide a detection signal at least 5-, 10-, or 20-fold higher than a detection signal provided with other proteins when used in Western blots or other immunochemical assays. Preferably, antibodies that specifically bind to polypeptides of the

invention do not bind to other proteins in immunochemical assays at detectable levels and can immunoprecipitate the specific polypeptide from solution.

The invention also contemplates naturally occurring antibodies specific for a polypeptide of the invention. For example, serum antibodies to a polypeptide of the invention in a human population can be purified by methods well known in the art, e.g., by passing antiserum over a column to which the corresponding selected polypeptide or fusion protein is bound. The bound antibodies can then be eluted from the column, for example using a buffer with a high salt concentration.

In addition to the antibodies discussed above, the invention also contemplates genetically engineered antibodies, antibody derivatives (e.g., single chain antibodies, antibody fragments (e.g., Fab, etc.)), according to methods well known in the art.

Polynucleotides or Arrays for Diagnostics

Polynucleotide arrays provide a high throughput technique that can assay a large number of polynucleotide sequences in a sample. This technology can be used as a diagnostic and as a tool to test for differential expression, e.g., to determine function of an encoded protein. Arrays can be created by spotting polynucleotide probes onto a substrate (e.g., glass, nitrocellulose, etc.) in a two-dimensional matrix or array having bound probes. The probes can be bound to the substrate by either covalent bonds or by non-specific interactions, such as hydrophobic interactions. Samples of polynucleotides can be detectably labeled (e.g., using radioactive or fluorescent labels) and then hybridized to the probes. Double stranded polynucleotides, comprising the labeled sample polynucleotides bound to probe polynucleotides, can be detected once the unbound portion of the sample is washed away. Techniques for constructing arrays and methods of using these arrays are described in EP 799 897; WO 97/29212; WO 97/27317; EP 785 280; WO 97/02357; USPN 5,593,839; USPN 5,578,832; EP 728 520; USPN 5,599,695; EP 721 016; USPN 5,556,752; WO 95/22058; and USPN 5,631,734. Arrays can be used to, for example, examine differential expression of genes and can be used to determine gene function. For example, arrays can be used to detect differential expression of a polynucleotide between a test cell and control cell (e.g., cancer cells and normal cells). For example, high expression of a particular message in a cancer cell, which is not observed in a corresponding normal cell, can indicate a cancer specific gene product. Exemplary uses of arrays are further described in, for example, Pappalarado *et al.*, *Sem. Radiation Oncol.* (1998) 8:217; and Ramsay *Nature Biotechnol.* (1998) 16:40.

Differential Expression in Diagnosis

The polynucleotides of the invention can also be used to detect differences in expression levels between two cells, e.g., as a method to identify abnormal or diseased tissue in a human. For polynucleotides corresponding to profiles of protein families, the choice of tissue can be selected according to the putative biological function. In general, the expression of a gene corresponding to

a specific polynucleotide is compared between a first tissue that is suspected of being diseased and a second, normal tissue of the human. The tissue suspected of being abnormal or diseased can be derived from a different tissue type of the human; but preferably it is derived from the same tissue type; for example an intestinal polyp or other abnormal growth should be compared with normal intestinal tissue. The normal tissue can be the same tissue as that of the test sample, or any normal tissue of the patient, especially those that express the polynucleotide-related gene of interest (*e.g.*, brain, thymus, testis, heart, prostate, placenta, spleen, small intestine, skeletal muscle, pancreas, and the mucosal lining of the colon). A difference between the polynucleotide-related gene, mRNA, or protein in the two tissues which are compared, for example in molecular weight, amino acid or nucleotide sequence, or relative abundance, indicates a change in the gene, or a gene which regulates it, in the tissue of the human that was suspected of being diseased. Examples of detection of differential expression and its use in diagnosis of cancer are described in USPNs 5,688,641 and 5,677,125.

A genetic predisposition to disease in a human can also be detected by comparing expression levels of an mRNA or protein corresponding to a polynucleotide of the invention in a fetal tissue with levels associated in normal fetal tissue. Fetal tissues that are used for this purpose include, but are not limited to, amniotic fluid, chorionic villi, blood, and the blastomere of an in vitro-fertilized embryo. The comparable normal polynucleotide-related gene is obtained from any tissue. The mRNA or protein is obtained from a normal tissue of a human in which the polynucleotide-related gene is expressed. Differences such as alterations in the nucleotide sequence or size of the same product of the fetal polynucleotide-related gene or mRNA, or alterations in the molecular weight, amino acid sequence, or relative abundance of fetal protein, can indicate a germline mutation in the polynucleotide-related gene of the fetus, which indicates a genetic predisposition to disease. In general, diagnostic, prognostic, and other methods of the invention based on differential expression involve detection of a level or amount of a gene product, particularly a differentially expressed gene product, in a test sample obtained from a patient suspected of having or being susceptible to a disease (*e.g.*, breast cancer, lung cancer, colon cancer and/or metastatic forms thereof), and comparing the detected levels to those levels found in normal cells (*e.g.*, cells substantially unaffected by cancer) and/or other control cells (*e.g.*, to differentiate a cancerous cell from a cell affected by dysplasia). Furthermore, the severity of the disease can be assessed by comparing the detected levels of a differentially expressed gene product with those levels detected in samples representing the levels of differentially gene product associated with varying degrees of severity of disease. It should be noted that use of the term "diagnostic" herein is not necessarily meant to exclude "prognostic" or "prognosis," but rather is used as a matter of convenience.

The term "differentially expressed gene" is generally intended to encompass a polynucleotide that can, for example, include an open reading frame encoding a gene product (*e.g.*, a polypeptide), and/or introns of such genes and adjacent 5' and 3' non-coding nucleotide sequences involved in the regulation of expression, up to about 20 kb beyond the coding region, but possibly further in either direction. The gene can be introduced into an appropriate vector for extrachromosomal maintenance or for integration into a host genome. In general, a difference in expression level associated with a decrease in expression level of at least about 25%, usually at least about 50% to 75%, more usually at least about 90% or more is indicative of a differentially expressed gene of interest, *i.e.*, a gene that is underexpressed or down-regulated in the test sample relative to a control sample. Furthermore, a difference in expression level associated with an increase in expression of at least about 25%, usually at least about 50% to 75%, more usually at least about 90% and can be at least about 1 1/2-fold, usually at least about 2-fold to about 10-fold, and can be about 100-fold to about 1,000-fold increase relative to a control sample is indicative of a differentially expressed gene of interest, *i.e.*, an overexpressed or up-regulated gene.

"Differentially expressed polynucleotide" as used herein means a nucleic acid molecule (RNA or DNA) comprising a sequence that represents a differentially expressed gene, *e.g.*, the differentially expressed polynucleotide comprises a sequence (*e.g.*, an open reading frame encoding a gene product) that uniquely identifies a differentially expressed gene so that detection of the differentially expressed polynucleotide in a sample is correlated with the presence of a differentially expressed gene in a sample. "Differentially expressed polynucleotides" is also meant to encompass fragments of the disclosed polynucleotides, *e.g.*, fragments retaining biological activity, as well as nucleic acids homologous, substantially similar, or substantially identical (*e.g.*, having about 90% sequence identity) to the disclosed polynucleotides.

"Diagnosis" as used herein generally includes determination of a subject's susceptibility to a disease or disorder, determination as to whether a subject is presently affected by a disease or disorder, as well as to the prognosis of a subject affected by a disease or disorder (*e.g.*, identification of pre-metastatic or metastatic cancerous states, stages of cancer, or responsiveness of cancer to therapy). The present invention particularly encompasses diagnosis of subjects in the context of breast cancer (*e.g.*, carcinoma in situ (*e.g.*, ductal carcinoma in situ), estrogen receptor (ER)-positive breast cancer, ER-negative breast cancer, or other forms and/or stages of breast cancer), lung cancer (*e.g.*, small cell carcinoma, non-small cell carcinoma, mesothelioma, and other forms and/or stages of lung cancer), and colon cancer (*e.g.*, adenomatous polyp, colorectal carcinoma, and other forms and/or stages of colon cancer).

"Sample" or "biological sample" as used throughout here are generally meant to refer to samples of biological fluids or tissues, particularly samples obtained from tissues, especially from cells of the type associated with the disease for which the diagnostic application is designed (*e.g.*,

ductal adenocarcinoma), and the like. "Samples" is also meant to encompass derivatives and fractions of such samples (e.g., cell lysates). Where the sample is solid tissue, the cells of the tissue can be dissociated or tissue sections can be analyzed.

Methods of the subject invention useful in diagnosis or prognosis typically involve
5 comparison of the abundance of a selected differentially expressed gene product in a sample of interest with that of a control to determine any relative differences in the expression of the gene product, where the difference can be measured qualitatively and/or quantitatively. Quantitation can be accomplished, for example, by comparing the level of expression product detected in the sample with the amounts of product present in a standard curve. A comparison can be made visually; by
10 using a technique such as densitometry, with or without computerized assistance; by preparing a representative library of cDNA clones of mRNA isolated from a test sample, sequencing the clones in the library to determine that number of cDNA clones corresponding to the same gene product, and analyzing the number of clones corresponding to that same gene product relative to the number of clones of the same gene product in a control sample; or by using an array to detect relative levels
15 of hybridization to a selected sequence or set of sequences, and comparing the hybridization pattern to that of a control. The differences in expression are then correlated with the presence or absence of an abnormal expression pattern. A variety of different methods for determining the nucleic acid abundance in a sample are known to those of skill in the art (see, e.g., WO 97/27317).

In general, diagnostic assays of the invention involve detection of a gene product of a the
20 polynucleotide sequence (e.g., mRNA or polypeptide) that corresponds to a sequence of SEQ ID NOS:1-316. The patient from whom the sample is obtained can be apparently healthy, susceptible to disease (e.g., as determined by family history or exposure to certain environmental factors), or can already be identified as having a condition in which altered expression of a gene product of the invention is implicated.

25 Diagnosis can be determined based on detected gene product expression levels of a gene product encoded by at least one, preferably at least two or more, at least 3 or more, or at least 4 or more of the polynucleotides having a sequence set forth in SEQ ID NOS:1-316, and can involve detection of expression of genes corresponding to all of SEQ ID NOS:1-316 and/or additional sequences that can serve as additional diagnostic markers and/or reference sequences. Where the
30 diagnostic method is designed to detect the presence or susceptibility of a patient to cancer, the assay preferably involves detection of a gene product encoded by a gene corresponding to a polynucleotide that is differentially expressed in cancer. Examples of such differentially expressed polynucleotides are described in the Examples below. Given the provided polynucleotides and information regarding their relative expression levels provided herein, assays using such
35 polynucleotides and detection of their expression levels in diagnosis and prognosis will be readily apparent to the ordinarily skilled artisan.

Any of a variety of detectable labels can be used in connection with the various embodiments of the diagnostic methods of the invention. Suitable detectable labels include fluorochromes, (e.g. fluorescein isothiocyanate (FITC), rhodamine, Texas Red, phycoerythrin, allophycocyanin, 6-carboxyfluorescein (6-FAM), 2',7'-dimethoxy-4',5'-dichloro-6-carboxyfluorescein, 6-carboxy-X-rhodamine (ROX), 6-carboxy-2',4',7',4,7-hexachlorofluorescein (HEX), 5-carboxyfluorescein (5-FAM) or N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA)), radioactive labels, (e.g. ^{32}P , ^{35}S , ^3H , etc.), and the like. The detectable label can involve a two stage systems (e.g., biotin-avidin, hapten-anti-hapten antibody, etc.)

Reagents specific for the polynucleotides and polypeptides of the invention, such as antibodies and nucleotide probes, can be supplied in a kit for detecting the presence of an expression product in a biological sample. The kit can also contain buffers or labeling components, as well as instructions for using the reagents to detect and quantify expression products in the biological sample. Exemplary embodiments of the diagnostic methods of the invention are described below in more detail.

Polypeptide detection in diagnosis. In one embodiment, the test sample is assayed for the level of a differentially expressed polypeptide. Diagnosis can be accomplished using any of a number of methods to determine the absence or presence or altered amounts of the differentially expressed polypeptide in the test sample. For example, detection can utilize staining of cells or histological sections with labeled antibodies, performed in accordance with conventional methods. Cells can be permeabilized to stain cytoplasmic molecules. In general, antibodies that specifically bind a differentially expressed polypeptide of the invention are added to a sample, and incubated for a period of time sufficient to allow binding to the epitope, usually at least about 10 minutes. The antibody can be detectably labeled for direct detection (e.g., using radioisotopes, enzymes, fluorescers, chemilumescers, and the like), or can be used in conjunction with a second stage antibody or reagent to detect binding (e.g., biotin with horseradish peroxidase-conjugated avidin, a secondary antibody conjugated to a fluorescent compound, e.g. fluorescein, rhodamine, Texas red, etc.). The absence or presence of antibody binding can be determined by various methods, including flow cytometry of dissociated cells, microscopy, radiography, scintillation counting, etc. Any suitable alternative methods can of qualitative or quantitative detection of levels or amounts of differentially expressed polypeptide can be used, for example ELISA, western blot, immunoprecipitation, radioimmunoassay, etc.

mRNA detection. The diagnostic methods of the invention can also or alternatively involve detection of mRNA encoded by a gene corresponding to a differentially expressed polynucleotides of the invention. Any suitable qualitative or quantitative methods known in the art for detecting specific mRNAs can be used. mRNA can be detected by, for example, *in situ* hybridization in tissue sections, by reverse transcriptase-PCR, or in Northern blots containing poly A+ mRNA. One

of skill in the art can readily use these methods to determine differences in the size or amount of mRNA transcripts between two samples. mRNA expression levels in a sample can also be determined by generation of a library of expressed sequence tags (ESTs) from the sample, where the EST library is representative of sequences present in the sample (Adams, et al., (1991) *Science* 252:1651). Enumeration of the relative representation of ESTs within the library can be used to approximate the relative representation of the gene transcript within the starting sample. The results of EST analysis of a test sample can then be compared to EST analysis of a reference sample to determine the relative expression levels of a selected polynucleotide, particularly a polynucleotide corresponding to one or more of the differentially expressed genes described herein. Alternatively, gene expression in a test sample can be performed using serial analysis of gene expression (SAGE) methodology (e.g., Velculescu et al., *Science* (1995) 270:484) or differential display (DD) methodology (see, e.g., U.S. 5,776,683; and U.S. 5,807,680).

Alternatively, gene expression can be analyzed using hybridization analysis. Oligonucleotides or cDNA can be used to selectively identify or capture DNA or RNA of specific sequence composition, and the amount of RNA or cDNA hybridized to a known capture sequence determined qualitatively or quantitatively, to provide information about the relative representation of a particular message within the pool of cellular messages in a sample. Hybridization analysis can be designed to allow for concurrent screening of the relative expression of hundreds to thousands of genes by using, for example, array-based technologies having high density formats, including filters, microscope slides, or microchips, or solution-based technologies that use spectroscopic analysis (e.g., mass spectrometry). One exemplary use of arrays in the diagnostic methods of the invention is described below in more detail.

Use of a single gene in diagnostic applications. The diagnostic methods of the invention can focus on the expression of a single differentially expressed gene. For example, the diagnostic method can involve detecting a differentially expressed gene, or a polymorphism of such a gene (e.g., a polymorphism in an coding region or control region), that is associated with disease. Disease-associated polymorphisms can include deletion or truncation of the gene, mutations that alter expression level and/or affect activity of the encoded protein, etc.

A number of methods are available for analyzing nucleic acids for the presence of a specific sequence, e.g. a disease associated polymorphism. Where large amounts of DNA are available, genomic DNA is used directly. Alternatively, the region of interest is cloned into a suitable vector and grown in sufficient quantity for analysis. Cells that express a differentially expressed gene can be used as a source of mRNA, which can be assayed directly or reverse transcribed into cDNA for analysis. The nucleic acid can be amplified by conventional techniques, such as the polymerase chain reaction (PCR), to provide sufficient amounts for analysis, and a detectable label can be included in the amplification reaction (e.g., using a detectably labeled

primer or detectably labeled oligonucleotides) to facilitate detection. Alternatively, various methods are also known in the art that utilize oligonucleotide ligation as a means of detecting polymorphisms, see e.g., Riley *et al.*, *Nucl. Acids Res.* (1990) 18:2887; and Delahunty *et al.*, *Am. J. Hum. Genet.* (1996) 58:1239.

5 The amplified or cloned sample nucleic acid can be analyzed by one of a number of methods known in the art. The nucleic acid can be sequenced by dideoxy or other methods, and the sequence of bases compared to a selected sequence, e.g., to a wild-type sequence. Hybridization with the polymorphic or variant sequence can also be used to determine its presence in a sample (e.g., by Southern blot, dot blot, *etc.*). The hybridization pattern of a polymorphic or variant
10 sequence and a control sequence to an array of oligonucleotide probes immobilized on a solid support, as described in US 5,445,934, or in WO 95/35505, can also be used as a means of identifying polymorphic or variant sequences associated with disease. Single strand conformational polymorphism (SSCP) analysis, denaturing gradient gel electrophoresis (DGGE), and heteroduplex analysis in gel matrices are used to detect conformational changes created by DNA sequence
15 variation as alterations in electrophoretic mobility. Alternatively, where a polymorphism creates or destroys a recognition site for a restriction endonuclease, the sample is digested with that endonuclease, and the products size fractionated to determine whether the fragment was digested. Fractionation is performed by gel or capillary electrophoresis, particularly acrylamide or agarose gels.

20 Screening for mutations in a gene can be based on the functional or antigenic characteristics of the protein. Protein truncation assays are useful in detecting deletions that can affect the biological activity of the protein. Various immunoassays designed to detect polymorphisms in proteins can be used in screening. Where many diverse genetic mutations lead to a particular disease phenotype, functional protein assays have proven to be effective screening
25 tools. The activity of the encoded protein can be determined by comparison with the wild-type protein.

Pattern matching in diagnosis using arrays. In another embodiment, the diagnostic and/or prognostic methods of the invention involve detection of expression of a selected set of genes in a test sample to produce a test expression pattern (TEP). The TEP is compared to a reference
30 expression pattern (REP), which is generated by detection of expression of the selected set of genes in a reference sample (e.g., a positive or negative control sample). The selected set of genes includes at least one of the genes of the invention, which genes correspond to the polynucleotide sequences of SEQ ID NOS:1-316. Of particular interest is a selected set of genes that includes gene differentially expressed in the disease for which the test sample is to be screened.

35 "Reference sequences" or "reference polynucleotides" as used herein in the context of differential gene expression analysis and diagnosis/prognosis refers to a selected set of

polynucleotides, which selected set includes at least one or more of the differentially expressed polynucleotides described herein. A plurality of reference sequences, preferably comprising positive and negative control sequences, can be included as reference sequences. Additional suitable reference sequences are found in GenBank, Unigene, and other nucleotide sequence
5 databases (including, *e.g.*, expressed sequence tag (EST), partial, and full-length sequences).

"Reference array" means an array having reference sequences for use in hybridization with a sample, where the reference sequences include all, at least one of, or any subset of the differentially expressed polynucleotides described herein. Usually such an array will include at least 3 different reference sequences, and can include any one or all of the provided differentially
10 expressed sequences. Arrays of interest can further comprise sequences, including polymorphisms, of other genetic sequences, particularly other sequences of interest for screening for a disease or disorder (*e.g.*, cancer, dysplasia, or other related or unrelated diseases, disorders, or conditions). The oligonucleotide sequence on the array will usually be at least about 12 nt in length, and can be of about the length of the provided sequences, or can extend into the flanking regions to generate
15 fragments of 100 nt to 200 nt in length or more. Reference arrays can be produced according to any suitable methods known in the art. For example, methods of producing large arrays of oligonucleotides are described in U.S. 5,134,854, and U.S. 5,445,934 using light-directed synthesis techniques. Using a computer controlled system, a heterogeneous array of monomers is converted, through simultaneous coupling at a number of reaction sites, into a heterogeneous array of
20 polymers. Alternatively, microarrays are generated by deposition of pre-synthesized oligonucleotides onto a solid substrate, for example as described in PCT published application no. WO 95/35505.

A "reference expression pattern" or "REP" as used herein refers to the relative levels of expression of a selected set of genes, particularly of differentially expressed genes, that is
25 associated with a selected cell type, *e.g.*, a normal cell, a cancerous cell, a cell exposed to an environmental stimulus, and the like. A "test expression pattern" or "TEP" refers to relative levels of expression of a selected set of genes, particularly of differentially expressed genes, in a test sample (*e.g.*, a cell of unknown or suspected disease state, from which mRNA is isolated).

REPs can be generated in a variety of ways according to methods well known in the art.
30 For example, REPs can be generated by hybridizing a control sample to an array having a selected set of polynucleotides (particularly a selected set of differentially expressed polynucleotides), acquiring the hybridization data from the array, and storing the data in a format that allows for ready comparison of the REP with a TEP. Alternatively, all expressed sequences in a control sample can be isolated and sequenced, *e.g.*, by isolating mRNA from a control sample, converting
35 the mRNA into cDNA, and sequencing the cDNA. The resulting sequence information roughly or precisely reflects the identity and relative number of expressed sequences in the sample. The

sequence information can then be stored in a format (e.g., a computer-readable format) that allows for ready comparison of the REP with a TEP. The REP can be normalized prior to or after data storage, and/or can be processed to selectively remove sequences of expressed genes that are of less interest or that might complicate analysis (e.g., some or all of the sequences associated with housekeeping genes can be eliminated from REP data).

TEPs can be generated in a manner similar to REPs, e.g., by hybridizing a test sample to an array having a selected set of polynucleotides, particularly a selected set of differentially expressed polynucleotides, acquiring the hybridization data from the array, and storing the data in a format that allows for ready comparison of the TEP with a REP. The REP and TEP to be used in a comparison can be generated simultaneously, or the TEP can be compared to previously generated and stored REPs.

In one embodiment of the invention, comparison of a TEP with a REP involves hybridizing a test sample with a reference array, where the reference array has one or more reference sequences for use in hybridization with a sample. The reference sequences include all, at least one of, or any subset of the differentially expressed polynucleotides described herein. Hybridization data for the test sample is acquired, the data normalized, and the produced TEP compared with a REP generated using an array having the same or similar selected set of differentially expressed polynucleotides. Probes that correspond to sequences differentially expressed between the two samples will show decreased or increased hybridization efficiency for one of the samples relative to the other.

Methods for collection of data from hybridization of samples with a reference arrays are well known in the art. For example, the polynucleotides of the reference and test samples can be generated using a detectable fluorescent label, and hybridization of the polynucleotides in the samples detected by scanning the microarrays for the presence of the detectable label using, for example, a microscope and light source for directing light at a substrate. A photon counter detects fluorescence from the substrate, while an x-y translation stage varies the location of the substrate. A confocal detection device that can be used in the subject methods is described in USPN 5,631,734. A scanning laser microscope is described in Shalon et al., *Genome Res.* (1996) 6:639. A scan, using the appropriate excitation line, is performed for each fluorophore used. The digital images generated from the scan are then combined for subsequent analysis. For any particular array element, the ratio of the fluorescent signal from one sample (e.g., a test sample) is compared to the fluorescent signal from another sample (e.g., a reference sample), and the relative signal intensity determined.

Methods for analyzing the data collected from hybridization to arrays are well known in the art. For example, where detection of hybridization involves a fluorescent label, data analysis can include the steps of determining fluorescent intensity as a function of substrate position from the data collected, removing outliers, i.e. data deviating from a predetermined statistical distribution,

and calculating the relative binding affinity of the targets from the remaining data. The resulting data can be displayed as an image with the intensity in each region varying according to the binding affinity between targets and probes.

In general, the test sample is classified as having a gene expression profile corresponding to that associated with a disease or non-disease state by comparing the TEP generated from the test sample to one or more REPs generated from reference samples (*e.g.*, from samples associated with cancer or specific stages of cancer, dysplasia, samples affected by a disease other than cancer, normal samples, *etc.*). The criteria for a match or a substantial match between a TEP and a REP include expression of the same or substantially the same set of reference genes, as well as expression of these reference genes at substantially the same levels (*e.g.*, no significant difference between the samples for a signal associated with a selected reference sequence after normalization of the samples, or at least no greater than about 25% to about 40% difference in signal strength for a given reference sequence. In general, a pattern match between a TEP and a REP includes a match in expression, preferably a match in qualitative or quantitative expression level, of at least one of, all or any subset of the differentially expressed genes of the invention.

Pattern matching can be performed manually, or can be performed using a computer program. Methods for preparation of substrate matrices (*e.g.*, arrays), design of oligonucleotides for use with such matrices, labeling of probes, hybridization conditions, scanning of hybridized matrices, and analysis of patterns generated, including comparison analysis, are described in, for example, U.S. 5,800,992.

Diagnosis, Prognosis and Management of Cancer

The polynucleotides of the invention and their gene products are of particular interest as genetic or biochemical markers (*e.g.*, in blood or tissues) that will detect the earliest changes along the carcinogenesis pathway and/or to monitor the efficacy of various therapies and preventive interventions. For example, the level of expression of certain polynucleotides can be indicative of a poorer prognosis, and therefore warrant more aggressive chemo- or radio-therapy for a patient or vice versa. The correlation of novel surrogate tumor specific features with response to treatment and outcome in patients can define prognostic indicators that allow the design of tailored therapy based on the molecular profile of the tumor. These therapies include antibody targeting and gene therapy. Determining expression of certain polynucleotides and comparison of a patient's profile with known expression in normal tissue and variants of the disease allows a determination of the best possible treatment for a patient, both in terms of specificity of treatment and in terms of comfort level of the patient. Surrogate tumor markers, such as polynucleotide expression, can also be used to better classify, and thus diagnose and treat, different forms and disease states of cancer. Two classifications widely used in oncology that can benefit from identification of the expression

levels of the polynucleotides of the invention are staging of the cancerous disorder, and grading the nature of the cancerous tissue.

The polynucleotides of the invention can be useful to monitor patients having or susceptible to cancer to detect potentially malignant events at a molecular level before they are detectable at a gross morphological level. Furthermore, a polynucleotide of the invention identified as important for one type of cancer can also have implications for development or risk of development of other types of cancer, e.g., where a polynucleotide is differentially expressed across various cancer types. Thus, for example, expression of a polynucleotide that has clinical implications for metastatic colon cancer can also have clinical implications for stomach cancer or endometrial cancer.

Staging. Staging is a process used by physicians to describe how advanced the cancerous state is in a patient. Staging assists the physician in determining a prognosis, planning treatment and evaluating the results of such treatment. Staging systems vary with the types of cancer, but generally involve the following "TNM" system: the type of tumor, indicated by T; whether the cancer has metastasized to nearby lymph nodes, indicated by N; and whether the cancer has metastasized to more distant parts of the body, indicated by M. Generally, if a cancer is only detectable in the area of the primary lesion without having spread to any lymph nodes it is called Stage I. If it has spread only to the closest lymph nodes, it is called Stage II. In Stage III, the cancer has generally spread to the lymph nodes in near proximity to the site of the primary lesion. Cancers that have spread to a distant part of the body, such as the liver, bone, brain or other site, are Stage IV, the most advanced stage.

The polynucleotides of the invention can facilitate fine-tuning of the staging process by identifying markers for the aggressivity of a cancer, e.g. the metastatic potential, as well as the presence in different areas of the body. Thus, a Stage II cancer with a polynucleotide signifying a high metastatic potential cancer can be used to change a borderline Stage II tumor to a Stage III tumor, justifying more aggressive therapy. Conversely, the presence of a polynucleotide signifying a lower metastatic potential allows more conservative staging of a tumor.

Grading of cancers. Grade is a term used to describe how closely a tumor resembles normal tissue of its same type. The microscopic appearance of a tumor is used to identify tumor grade based on parameters such as cell morphology, cellular organization, and other markers of differentiation. As a general rule, the grade of a tumor corresponds to its rate of growth or aggressiveness, with undifferentiated or high-grade tumors being more aggressive than well differentiated or low-grade tumors. The following guidelines are generally used for grading tumors: 1) GX Grade cannot be assessed; 2) G1 Well differentiated; G2 Moderately well differentiated; 3) G3 Poorly differentiated; 4) G4 Undifferentiated. The polynucleotides of the invention can be especially valuable in determining the grade of the tumor, as they not only can aid in determining

the differentiation status of the cells of a tumor, they can also identify factors other than differentiation that are valuable in determining the aggressiveness of a tumor, such as metastatic potential.

Detection of lung cancer. The polynucleotides of the invention can be used to detect lung cancer in a subject. Although there are more than a dozen different kinds of lung cancer, the two main types of lung cancer are small cell and nonsmall cell, which encompass about 90% of all lung cancer cases. Small cell carcinoma (also called oat cell carcinoma) usually starts in one of the larger bronchial tubes, grows fairly rapidly, and is likely to be large by the time of diagnosis. Nonsmall cell lung cancer (NSCLC) is made up of three general subtypes of lung cancer. Epidermoid carcinoma (also called squamous cell carcinoma) usually starts in one of the larger bronchial tubes and grows relatively slowly. The size of these tumors can range from very small to quite large. Adenocarcinoma starts growing near the outside surface of the lung and can vary in both size and growth rate. Some slowly growing adenocarcinomas are described as alveolar cell cancer. Large cell carcinoma starts near the surface of the lung, grows rapidly, and the growth is usually fairly large when diagnosed. Other less common forms of lung cancer are carcinoid, cylindroma, mucoepidermoid, and malignant mesothelioma.

The polynucleotides of the invention, e.g., polynucleotides differentially expressed in normal cells versus cancerous lung cells (e.g., tumor cells of high or low metastatic potential) or between types of cancerous lung cells (e.g., high metastatic versus low metastatic), can be used to distinguish types of lung cancer as well as identifying traits specific to a certain patient's cancer and selecting an appropriate therapy. For example, if the patient's biopsy expresses a polynucleotide that is associated with a low metastatic potential, it may justify leaving a larger portion of the patient's lung in surgery to remove the lesion. Alternatively, a smaller lesion with expression of a polynucleotide that is associated with high metastatic potential may justify a more radical removal of lung tissue and/or the surrounding lymph nodes, even if no metastasis can be identified through pathological examination.

Detection of breast cancer. The majority of breast cancers are adenocarcinomas subtypes, which can be summarized as follows: 1) ductal carcinoma in situ (DCIS), including comedocarcinoma; 2) infiltrating (or invasive) ductal carcinoma (IDC); 3) lobular carcinoma in situ (LCIS); 4) infiltrating (or invasive) lobular carcinoma (ILC); 5) inflammatory breast cancer; 6) medullary carcinoma; 7) mucinous carcinoma; 8) Paget's disease of the nipple; 9) Phyllodes tumor; and 10) tubular carcinoma;

The expression of polynucleotides of the invention can be used in the diagnosis and management of breast cancer, as well as to distinguish between types of breast cancer. Detection of breast cancer can be determined using expression levels of any of the appropriate polynucleotides of the invention, either alone or in combination. Determination of the aggressive nature and/or the

metastatic potential of a breast cancer can also be determined by comparing levels of one or more polynucleotides of the invention and comparing levels of another sequence known to vary in cancerous tissue, e.g. ER expression. In addition, development of breast cancer can be detected by examining the ratio of expression of a differentially expressed polynucleotide to the levels of steroid hormones (e.g., testosterone or estrogen) or to other hormones (e.g., growth hormone, insulin). Thus expression of specific marker polynucleotides can be used to discriminate between normal and cancerous breast tissue, to discriminate between breast cancers with different cells of origin, to discriminate between breast cancers with different potential metastatic rates, etc.

Detection of colon cancer. The polynucleotides of the invention exhibiting the appropriate expression pattern can be used to detect colon cancer in a subject. Colorectal cancer is one of the most common neoplasms in humans and perhaps the most frequent form of hereditary neoplasia. Prevention and early detection are key factors in controlling and curing colorectal cancer. Colorectal cancer begins as polyps, which are small, benign growths of cells that form on the inner lining of the colon. Over a period of several years, some of these polyps accumulate additional mutations and become cancerous. Multiple familial colorectal cancer disorders have been identified, which are summarized as follows: 1) Familial adenomatous polyposis (FAP); 2) Gardner's syndrome; 3) Hereditary nonpolyposis colon cancer (HNPCC); and 4) Familial colorectal cancer in Ashkenazi Jews. The expression of appropriate polynucleotides of the invention can be used in the diagnosis, prognosis and management of colorectal cancer. Detection of colon cancer can be determined using expression levels of any of these sequences alone or in combination with the levels of expression. Determination of the aggressive nature and/or the metastatic potential of a colon cancer can be determined by comparing levels of one or more polynucleotides of the invention and comparing total levels of another sequence known to vary in cancerous tissue, e.g., expression of p53, DCC ras, or FAP (see, e.g., Fearon ER, *et al.*, *Cell* (1990) 61(5):759; Hamilton SR *et al.*, *Cancer* (1993) 72:957; Bodmer W, *et al.*, *Nat Genet.* (1994) 4(3):217; Fearon ER, *Ann N Y Acad Sci.* (1995) 768:101). For example, development of colon cancer can be detected by examining the ratio of any of the polynucleotides of the invention to the levels of oncogenes (e.g. ras) or tumor suppressor genes (e.g. FAP or p53). Thus expression of specific marker polynucleotides can be used to discriminate between normal and cancerous colon tissue, to discriminate between colon cancers with different cells of origin, to discriminate between colon cancers with different potential metastatic rates, etc.

Detection of prostate cancer. The polynucleotides and their corresponding genes and gene products exhibiting the appropriate differential expression pattern can be used to detect prostate cancer in a subject. Over 95% of primary prostate cancers are adenocarcinomas. Signs and symptoms may include: frequent urination, especially at night, inability to urinate, trouble starting

or holding back urination, a weak or interrupted urine flow and frequent pain or stiffness in the lower back, hips or upper thighs.

Many of the signs and symptoms of prostate cancer can be caused by a variety of other non-cancerous conditions. For example, one common cause of many of these signs and symptoms is a condition called benign prostatic hypertrophy, or BPH. In BPH, the prostate gets bigger and may block the flow of urine or interfere with sexual function. The methods and compositions of the invention can be used to distinguish between prostate cancer and such non-cancerous conditions. The methods of the invention can be used in conjunction with conventional methods of diagnosis, e.g., digital rectal exam and/or detection of the level of prostate specific antigen (PSA), a substance produced and secreted by the prostate.

Use of Polynucleotides to Screen for Peptide Analogs and Antagonists

Polypeptides encoded by the instant polynucleotides and corresponding full length genes can be used to screen peptide libraries to identify binding partners, such as receptors, from among the encoded polypeptides. Peptide libraries can be synthesized according to methods known in the art (see, e.g., USPN 5,010,175, and WO 91/17823). Agonists or antagonists of the polypeptides if the invention can be screened using any available method known in the art, such as signal transduction, antibody binding, receptor binding, mitogenic assays, chemotaxis assays, etc. The assay conditions ideally should resemble the conditions under which the native activity is exhibited *in vivo*, that is, under physiologic pH, temperature, and ionic strength. Suitable agonists or antagonists will exhibit strong inhibition or enhancement of the native activity at concentrations that do not cause toxic side effects in the subject. Agonists or antagonists that compete for binding to the native polypeptide can require concentrations equal to or greater than the native concentration, while inhibitors capable of binding irreversibly to the polypeptide can be added in concentrations on the order of the native concentration.

Such screening and experimentation can lead to identification of a novel polypeptide binding partner, such as a receptor, encoded by a gene or a cDNA corresponding to a polynucleotide of the invention, and at least one peptide agonist or antagonist of the novel binding partner. Such agonists and antagonists can be used to modulate, enhance, or inhibit receptor function in cells to which the receptor is native, or in cells that possess the receptor as a result of genetic engineering. Further, if the novel receptor shares biologically important characteristics with a known receptor, information about agonist/antagonist binding can facilitate development of improved agonists/antagonists of the known receptor.

Pharmaceutical Compositions and Therapeutic Uses

Pharmaceutical compositions of the invention can comprise polypeptides, antibodies, or polynucleotides (including antisense nucleotides and ribozymes) of the claimed invention in a therapeutically effective amount. The term "therapeutically effective amount" as used herein refers

to an amount of a therapeutic agent to treat, ameliorate, or prevent a desired disease or condition, or to exhibit a detectable therapeutic or preventative effect. The effect can be detected by, for example, chemical markers or antigen levels. Therapeutic effects also include reduction in physical symptoms, such as decreased body temperature. The precise effective amount for a subject will
5 depend upon the subject's size and health, the nature and extent of the condition, and the therapeutics or combination of therapeutics selected for administration. Thus, it is not useful to specify an exact effective amount in advance. However, the effective amount for a given situation is determined by routine experimentation and is within the judgment of the clinician. For purposes of the present invention, an effective dose will generally be from about 0.01 mg/ kg to 50 mg/kg or
10 0.05 mg/kg to about 10 mg/kg of the DNA constructs in the individual to which it is administered.

A pharmaceutical composition can also contain a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable carrier" refers to a carrier for administration of a therapeutic agent, such as antibodies or a polypeptide, genes, and other therapeutic agents. The term refers to any pharmaceutical carrier that does not itself induce the production of antibodies harmful to the
15 individual receiving the composition, and which can be administered without undue toxicity. Suitable carriers can be large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles. Such carriers are well known to those of ordinary skill in the art. Pharmaceutically acceptable carriers in therapeutic compositions can include liquids such
20 as water, saline, glycerol and ethanol. Auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, can also be present in such vehicles. Typically, the therapeutic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared. Liposomes are included within the definition of a pharmaceutically acceptable carrier.
25 Pharmaceutically acceptable salts can also be present in the pharmaceutical composition, e.g., mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. A thorough discussion of pharmaceutically acceptable excipients is available in *Remington's Pharmaceutical Sciences* (Mack Pub. Co., N.J. 1991).

Delivery Methods. Once formulated, the compositions of the invention can be
30 (1) administered directly to the subject (e.g., as polynucleotide or polypeptides); or (2) delivered ex vivo, to cells derived from the subject (e.g., as in *ex vivo* gene therapy). Direct delivery of the compositions will generally be accomplished by parenteral injection, e.g., subcutaneously; intraperitoneally, intravenously or intramuscularly, intratumoral or to the interstitial space of a
35 tissue. Other modes of administration include oral and pulmonary administration, suppositories,

and transdermal applications, needles, and gene guns or hyposprays. Dosage treatment can be a single dose schedule or a multiple dose schedule.

Methods for the ex vivo delivery and reimplantation of transformed cells into a subject are known in the art and described in *e.g.*, International Publication No. WO 93/14778. Examples of
5 cells useful in ex vivo applications include, for example, stem cells, particularly hematopoietic, lymph cells, macrophages, dendritic cells, or tumor cells. Generally, delivery of nucleic acids for both ex vivo and in vitro applications can be accomplished by, for example, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of
10 the DNA into nuclei, all well known in the art.

Once a gene corresponding to a polynucleotide of the invention has been found to correlate with a proliferative disorder, such as neoplasia, dysplasia, and hyperplasia, the disorder can be amenable to treatment by administration of a therapeutic agent based on the provided polynucleotide, corresponding polypeptide or other corresponding molecule (*e.g.*, antisense,
15 ribozyme, etc.).

The dose and the means of administration of the inventive pharmaceutical compositions are determined based on the specific qualities of the therapeutic composition, the condition, age, and weight of the patient, the progression of the disease, and other relevant factors. For example, administration of polynucleotide therapeutic compositions agents of the invention includes local or
20 systemic administration, including injection, oral administration, particle gun or catheterized administration, and topical administration. Preferably, the therapeutic polynucleotide composition contains an expression construct comprising a promoter operably linked to a polynucleotide of at least 12, 22, 25, 30, or 35 contiguous nt of the polynucleotide disclosed herein. Various methods can be used to administer the therapeutic composition directly to a specific site in the body. For
25 example, a small metastatic lesion is located and the therapeutic composition injected several times in several different locations within the body of tumor. Alternatively, arteries which serve a tumor are identified, and the therapeutic composition injected into such an artery, in order to deliver the composition directly into the tumor. A tumor that has a necrotic center is aspirated and the composition injected directly into the now empty center of the tumor. The antisense composition is
30 directly administered to the surface of the tumor, for example, by topical application of the composition. X-ray imaging is used to assist in certain of the above delivery methods.

Receptor-mediated targeted delivery of therapeutic compositions containing an antisense polynucleotide, subgenomic polynucleotides, or antibodies to specific tissues can also be used. Receptor-mediated DNA delivery techniques are described in, for example, Findeis *et al.*, *Trends*
35 *Biotechnol.* (1993) 11:202; Chiou *et al.*, *Gene Therapeutics: Methods And Applications Of Direct Gene Transfer* (J.A. Wolff, ed.) (1994); Wu *et al.*, *J. Biol. Chem.* (1988) 263:621; Wu *et al.*, *J. Biol.*

Chem. (1994) 269:542; Zenke *et al.*, *Proc. Natl. Acad. Sci. (USA)* (1990) 87:3655; Wu *et al.*, *J. Biol. Chem.* (1991) 266:338. Therapeutic compositions containing a polynucleotide are administered in a range of about 100 ng to about 200 mg of DNA for local administration in a gene therapy protocol. Concentration ranges of about 500 ng to about 50 mg, about 1 µg to about 2 mg, about 5 µg to about 500 µg, and about 20 µg to about 100 µg of DNA can also be used during a gene therapy protocol. Factors such as method of action (e.g., for enhancing or inhibiting levels of the encoded gene product) and efficacy of transformation and expression are considerations which will affect the dosage required for ultimate efficacy of the antisense subgenomic polynucleotides. Where greater expression is desired over a larger area of tissue, larger amounts of antisense subgenomic polynucleotides or the same amounts readministered in a successive protocol of administrations, or several administrations to different adjacent or close tissue portions of, for example, a tumor site, may be required to effect a positive therapeutic outcome. In all cases, routine experimentation in clinical trials will determine specific ranges for optimal therapeutic effect. For polynucleotide related genes encoding polypeptides or proteins with anti-inflammatory activity, suitable use, doses, and administration are described in USPN 5,654,173.

The therapeutic polynucleotides and polypeptides of the present invention can be delivered using gene delivery vehicles. The gene delivery vehicle can be of viral or non-viral origin (see generally, Jolly, *Cancer Gene Therapy* (1994) 1:51; Kimura, *Human Gene Therapy* (1994) 5:845; Connelly, *Human Gene Therapy* (1995) 1:185; and Kaplitt, *Nature Genetics* (1994) 6:148).

Expression of such coding sequences can be induced using endogenous mammalian or heterologous promoters. Expression of the coding sequence can be either constitutive or regulated.

Viral-based vectors for delivery of a desired polynucleotide and expression in a desired cell are well known in the art. Exemplary viral-based vehicles include, but are not limited to, recombinant retroviruses (see, e.g., WO 90/07936; WO 94/03622; WO 93/25698; WO 93/25234; USPN 5, 219,740; WO 93/11230; WO 93/10218; USPN 4,777,127; GB Patent No. 2,200,651; EP 0 345 242; and WO 91/02805), alphavirus-based vectors (e.g., Sindbis virus vectors, Semliki forest virus (ATCC VR-67; ATCC VR-1247), Ross River virus (ATCC VR-373; ATCC VR-1246) and Venezuelan equine encephalitis virus (ATCC VR-923; ATCC VR-1250; ATCC VR 1249; ATCC VR-532), and adeno-associated virus (AAV) vectors (see, e.g., WO 94/12649, WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984 and WO 95/00655). Administration of DNA linked to killed adenovirus as described in Curiel, *Hum. Gene Ther.* (1992) 3:147 can also be employed.

Non-viral delivery vehicles and methods can also be employed, including, but not limited to, polycationic condensed DNA linked or unlinked to killed adenovirus alone (see, e.g., Curiel, *Hum. Gene Ther.* (1992) 3:147); ligand-linked DNA (see, e.g., Wu, *J. Biol. Chem.* (1989) 264:16985); eukaryotic cell delivery vehicles (see, e.g., USPN 5,814,482; WO 95/07994; WO 96/17072; WO 95/30763; and WO 97/42338) and nucleic charge neutralization or fusion with

cell membranes. Naked DNA can also be employed. Exemplary naked DNA introduction methods are described in WO 90/11092 and USPN 5,580,859. Liposomes that can act as gene delivery vehicles are described in USPN 5,422,120; WO 95/13796; WO 94/23697; WO 91/14445; and EP 0524968. Additional approaches are described in Philip, *Mol. Cell Biol.* (1994) 14:2411, and in Woffendin, *Proc. Natl. Acad. Sci.* (1994) 91:1581

Further non-viral delivery suitable for use includes mechanical delivery systems such as the approach described in Woffendin *et al.*, *Proc. Natl. Acad. Sci. USA* (1994) 91(24):11581.

Moreover, the coding sequence and the product of expression of such can be delivered through deposition of photopolymerized hydrogel materials or use of ionizing radiation (see, e.g., USPN 5,206,152 and WO 92/11033). Other conventional methods for gene delivery that can be used for delivery of the coding sequence include, for example, use of hand-held gene transfer particle gun (see, e.g., USPN 5,149,655); use of ionizing radiation for activating transferred gene (see, e.g., USPN 5,206,152 and WO 92/11033).

The present invention will now be illustrated by reference to the following examples which set forth particularly advantageous embodiments. However, it should be noted that these embodiments are illustrative and are not to be construed as restricting the invention in any way.

EXAMPLES

The following examples are offered primarily for purposes of illustration. It will be readily apparent to those skilled in the art that the formulations, dosages, methods of administration, and other parameters of this invention may be further modified or substituted in various ways without departing from the spirit and scope of the invention.

Example 1: Source of Biological Materials and Overview of Novel Polynucleotides Expressed by the Biological Materials

cDNA libraries were constructed from mRNA isolated from the GRRpz or and WOca cells, which were provided by Dr. Donna M. Peehl, Department of Medicine, Stanford University School of Medicine. GRRpz cells were primary cells derived from normal prostate epithelium. The WOca cells were prostate epithelial cells derived from prostate cancer Gleason Grade 4+4.

Polynucleotides expressed by these cells were isolated and analyzed; the sequences of these polynucleotides were about 275-300 nucleotides in length.

The sequences of the isolated polynucleotides were first masked to eliminate low complexity sequences using the XBLAST masking program (Claverie "Effective Large-Scale Sequence Similarity Searches," In: Computer Methods for Macromolecular Sequence Analysis, Doolittle, ed., *Meth. Enzymol.* 266:212-227 Academic Press, NY, NY (1996); see particularly Claverie, in "Automated DNA Sequencing and Analysis Techniques" Adams *et al.*, eds., Chap. 36,

p. 267 Academic Press, San Diego, 1994 and Claverie *et al. Comput. Chem.* (1993) 17:191).

Generally, masking does not influence the final search results, except to eliminate sequences of relative little interest due to their low complexity, and to eliminate multiple "hits" based on similarity to repetitive regions common to multiple sequences, e.g., Alu repeats. The remaining
5 sequences were then used in a BLASTN vs. GenBank search; sequences that exhibited greater than 70% overlap, 99% identity, and a p value of less than 1×10^{-40} were discarded. Sequences from this search also were discarded if the inclusive parameters were met, but the sequence was ribosomal or vector-derived.

The resulting sequences from the previous search were classified into three groups (1, 2
10 and 3 below) and searched in a BLASTX vs. NRP (non-redundant proteins) database search: (1) unknown (no hits in the GenBank search), (2) weak similarity (greater than 45% identity and p value of less than 1×10^{-5}), and (3) high similarity (greater than 60% overlap, greater than 80% identity, and p value less than 1×10^{-5}). Sequences having greater than 70% overlap, greater than 99% identity, and p value of less than 1×10^{-40} were discarded.

The remaining sequences were classified as unknown (no hits), weak similarity, and high
15 similarity (parameters as above). Two searches were performed on these sequences. First, a BLAST vs. EST database search was performed and sequences with greater than 99% overlap, greater than 99% similarity and a p value of less than 1×10^{-40} were discarded. Sequences with a p value of less than 1×10^{-65} when compared to a database sequence of human origin were also
20 excluded. Second, a BLASTN vs. Patent GeneSeq database was performed and sequences having greater than 99% identity, p value less than 1×10^{-40} , and greater than 99% overlap were discarded.

The remaining sequences were subjected to screening using other rules and redundancies in the dataset. Sequences with a p value of less than 1×10^{-111} in relation to a database sequence of human origin were specifically excluded. The final result provided the 316 sequences listed as
25 SEQ ID NOS:1-316 in the accompanying Sequence Listing and summarized in Table 1 (inserted prior to claims). Each identified polynucleotide represents sequence from at least a partial mRNA transcript. Many of the sequences include the sequence ggcacgag at the 5' end; this sequence is a sequencing artifact and not part of the sequence of the polynucleotides of the invention.

Table 1 provides: 1) the SEQ ID NO ("SEQ ID") assigned to each sequence for use in the
30 present specification; 2) the Cluster Identification No. ("CLUSTER"); 3) the sequence name ("SEQ NAME") used as an internal identifier of the sequence; 4) the orientation of the sequence ("ORIENT"); 5) the name assigned to the clone from which the sequence was isolated ("CLONE ID"); and the name of the library from which the sequence was isolated ("LIBRARY"). CH22PRC indicates the sequence was isolated from Library 22; CH21PRN indicates the sequence was isolated
35 from Library 21. A description of the libraries is provided in Table 3 below. Because the provided polynucleotides represent partial mRNA transcripts, two or more polynucleotides of the invention

may represent different regions of the same mRNA transcript and the same gene. Thus, if two or more SEQ ID NOS: are identified as belonging to the same clone, then either sequence can be used to obtain the full-length mRNA or gene.

5 Example 2: Results of Public Database Search to Identify Function of Gene Products

SEQ ID NOS:1-316 were translated in all three reading frames, and the nucleotide sequences and translated amino acid sequences used as query sequences to search for homologous sequences in either the GenBank (nucleotide sequences) or Non-Redundant Protein (amino acid sequences) databases. Query and individual sequences were aligned using the BLAST 2.0
10 programs, available over the world wide web at a saite sponsored by the National Center for Biotechnology Information, which is supported by the National Library of Medicine and the National Institutes of Health (see also Altschul, et al. *Nucleic Acids Res.* (1997) 25:3389-3402). The sequences were masked to various extents to prevent searching of repetitive sequences or poly-A sequences, using the XBLAST program for masking low complexity as described above in
15 Example 1.

Table 2 (inserted before the claims) provide the alignment summaries having a p value of 1×10^{-2} or less indicating substantial homology between the sequences of the present invention and those of the indicated public databases. Specifically, Table 2 provides the SEQ ID NO of the query sequence, the accession number of the GenBank database entry of the homologous sequence, and
20 the p value of the alignment. Table 2 also provides the SEQ ID NO of the query sequence, the accession number of the Non-Redundant Protein database entry of the homologous sequence, and the p value of the alignment. The alignments provided in Table 2 are the best available alignment to a DNA or amino acid sequence at a time just prior to filing of the present specification. The activity of the polypeptide encoded by the SEQ ID NOS listed in Table 2 can be extrapolated to be
25 substantially the same or substantially similar to the activity of the reported nearest neighbor or closely related sequence. The accession number of the nearest neighbor is reported, providing a publicly available reference to the activities and functions exhibited by the nearest neighbor. The public information regarding the activities and functions of each of the nearest neighbor sequences is incorporated by reference in this application. Also incorporated by reference is all publicly
30 available information regarding the sequence, as well as the putative and actual activities and functions of the nearest neighbor sequences listed in Table 2 and their related sequences. The search program and database used for the alignment, as well as the calculation of the p value are also indicated.

Full length sequences or fragments of the polynucleotide sequences of the nearest
35 neighbors can be used as probes and primers to identify and isolate the full length sequence of the corresponding polynucleotide. The nearest neighbors can indicate a tissue or cell type to be used to

construct a library for the full-length sequences of the corresponding polynucleotides.

Example 3: Differential Expression of Polynucleotides of the Invention: Description of Libraries and

5 Detection of Differential Expression

The relative expression levels of the polynucleotides of the invention was assessed in several libraries prepared from various sources, including primary cells, cell lines and patient tissue samples. Table 3 provides a summary of these libraries, including the shortened library name (used hereafter), the mRNA source used to prepared the cDNA library, the "nickname" of the library that
10 is used in the tables below (in quotes), and the approximate number of clones in the library.

Table 3. Description of cDNA Libraries

Library (Lib#)	Description	Number of Clones in Library
1	Human Colon Cell Line Km12 L4: High Metastatic Potential (derived from Km12C)	308731
2	Human Colon Cell Line Km12C: Low Metastatic Potential	284771
3	Human Breast Cancer Cell Line MDA-MB-231: High Metastatic Potential; micro-mets in lung	326937
4	Human Breast Cancer Cell Line MCF7: Non Metastatic	318979
8	Human Lung Cancer Cell Line MV-522: High Metastatic Potential	223620
9	Human Lung Cancer Cell Line UCP-3: Low Metastatic Potential	312503
12	Human microvascular endothelial cells (HMVEC) - UNTREATED (PCR (OligodT) cDNA library)	41938
13	Human microvascular endothelial cells (HMVEC) - bFGF TREATED (PCR (OligodT) cDNA library)	42100
14	Human microvascular endothelial cells (HMVEC) - VEGF TREATED (PCR (OligodT) cDNA library)	42825
15	Normal Colon - UC#2 Patient (MICRODISSECTED PCR (OligodT) cDNA library)	282722
16	Colon Tumor - UC#2 Patient (MICRODISSECTED PCR (OligodT) cDNA library)	298831
17	Liver Metastasis from Colon Tumor of UC#2 Patient	303467

Library (Lib#)	Description	Number of Clones in Library
	(MICRODISSECTED PCR (OligodT) cDNA library)	
18	Normal Colon - UC#3 Patient (MICRODISSECTED PCR (OligodT) cDNA library)	36216
19	Colon Tumor - UC#3 Patient (MICRODISSECTED PCR (OligodT) cDNA library)	41388
20	Liver Metastasis from Colon Tumor of UC#3 Patient (MICRODISSECTED PCR (OligodT) cDNA library)	30956
21	GRRpz Cells derived from normal prostate epithelium	164801
22	WOca Cells derived from Gleason Grade 4 prostate cancer epithelium	162088
23-	Normal Lung Epithelium of Patient #1006 (MICRODISSECTED PCR (OligodT) cDNA library)	306198
24	Primary tumor, Large Cell Carcinoma of Patient #1006 (MICRODISSECTED PCR (OligodT) cDNA library)	309349

The KM12L4 cell line is derived from the KM12C cell line (Morikawa, et al., *Cancer Research* (1988) 48:6863). The KM12C cell line, which is poorly metastatic (low metastatic) was established in culture from a Dukes' stage B₂ surgical specimen (Morikawa *et al. Cancer Res.* (1988) 48:6863). The KML4-A is a highly metastatic subline derived from KM12C (Yeatman *et al. Nucl. Acids. Res.* (1995) 23:4007; Bao-Ling *et al. Proc. Annu. Meet. Am. Assoc. Cancer Res.* (1995) 21:3269). The KM12C and KM12C-derived cell lines (e.g., KM12L4, KM12L4-A, etc.) are well-recognized in the art as a model cell line for the study of colon cancer (see, e.g., Moriakawa *et al., supra*; Radinsky *et al. Clin. Cancer Res.* (1995) 1:19; Yeatman *et al., (1995) supra*; Yeatman *et al. Clin. Exp. Metastasis* (1996) 14:246). The MDA-MB-231 cell line (Brinkley *et al. Cancer Res.* (1980) 40:3118-3129) was originally isolated from pleural effusions (Cailleau, *J. Natl. Cancer. Inst.* (1974) 53:661), is of high metastatic potential, and forms poorly differentiated adenocarcinoma grade II in nude mice consistent with breast carcinoma.

The MCF7 cell line was derived from a pleural effusion of a breast adenocarcinoma and is non-metastatic. The MV-522 cell line is derived from a human lung carcinoma and is of high metastatic potential. The UCP-3 cell line is a low metastatic human lung carcinoma cell line; the MV-522 is a high metastatic variant of UCP-3. These cell lines are well-recognized in the art as models for the study of human breast and lung cancer (see, e.g., Chandrasekaran *et al., Cancer Res.* (1979) 39:870 (MDA-MB-231 and MCF-7); Gastpar *et al., J Med Chem* (1998) 41:4965 (MDA-

MB-231 and MCF-7); Ranson *et al.*, *Br J Cancer* (1998) 77:1586 (MDA-MB-231 and MCF-7); Kuang *et al.*, *Nucleic Acids Res* (1998) 26:1116 (MDA-MB-231 and MCF-7); Varki *et al.*, *Int J Cancer* (1987) 40:46 (UCP-3); Varki *et al.*, *Tumour Biol.* (1990) 11:327; (MV-522 and UCP-3); Varki *et al.*, *Anticancer Res.* (1990) 10:637; (MV-522); Kelner *et al.*, *Anticancer Res* (1995) 15:867 (MV-522); and Zhang *et al.*, *Anticancer Drugs* (1997) 8:696 (MV522)). The samples of libraries 15-20 are derived from two different patients (UC#2, and UC#3). The bFGF-treated HMVEC were prepared by incubation with bFGF at 10ng/ml for 2 hrs; the VEGF-treated HMVEC were prepared by incubation with 20ng/ml VEGF for 2 hrs. Following incubation with the respective growth factor, the cells were washed and lysis buffer added for RNA preparation. The GRRpz and WOca cells were provided by Dr. Donna M. Peehl, Department of Medicine, Stanford University School of Medicine. GRRpz cells were derived from normal prostate epithelium. The WOca cells are Gleason Grade 4 cell line.

Each of the libraries is composed of a collection of cDNA clones that in turn are representative of the mRNAs expressed in the indicated mRNA source. In order to facilitate the analysis of the millions of sequences in each library, the sequences were assigned to clusters. The concept of "cluster of clones" is derived from a sorting/grouping of cDNA clones based on their hybridization pattern to a panel of roughly 300 7bp oligonucleotide probes (see Drmanac *et al.*, *Genomics* (1996) 37(1):29). Random cDNA clones from a tissue library are hybridized at moderate stringency to 300 7bp oligonucleotides. Each oligonucleotide has some measure of specific hybridization to that specific clone. The combination of 300 of these measures of hybridization for 300 probes equals the "hybridization signature" for a specific clone. Clones with similar sequence will have similar hybridization signatures. By developing a sorting/grouping algorithm to analyze these signatures, groups of clones in a library can be identified and brought together computationally. These groups of clones are termed "clusters". Depending on the stringency of the selection in the algorithm (similar to the stringency of hybridization in a classic library cDNA screening protocol), the "purity" of each cluster can be controlled. For example, artifacts of clustering may occur in computational clustering just as artifacts can occur in "wet-lab" screening of a cDNA library with 400 bp cDNA fragments, at even the highest stringency. The stringency used in the implementation of cluster herein provides groups of clones that are in general from the same cDNA or closely related cDNAs. Closely related clones can be a result of different length clones of the same cDNA, closely related clones from highly related gene families, or splice variants of the same cDNA.

Differential expression for a selected cluster was assessed by first determining the number of cDNA clones corresponding to the selected cluster in the first library (Clones in 1st), and the determining the number of cDNA clones corresponding to the selected cluster in the second library (Clones in 2nd). Differential expression of the selected cluster in the first library relative to the

second library is expressed as a "ratio" of percent expression between the two libraries. In general, the "ratio" is calculated by: 1) calculating the percent expression of the selected cluster in the first library by dividing the number of clones corresponding to a selected cluster in the first library by the total number of clones analyzed from the first library; 2) calculating the percent expression of the selected cluster in the second library by dividing the number of clones corresponding to a selected cluster in a second library by the total number of clones analyzed from the second library; 3) dividing the calculated percent expression from the first library by the calculated percent expression from the second library. If the "number of clones" corresponding to a selected cluster in a library is zero, the value is set at 1 to aid in calculation. The formula used in calculating the ratio takes into account the "depth" of each of the libraries being compared, *i.e.*, the total number of clones analyzed in each library.

In general, a polynucleotide is said to be significantly differentially expressed between two samples when the ratio value is greater than at least about 2, preferably greater than at least about 3, more preferably greater than at least about 5, where the ratio value is calculated using the method described above. The significance of differential expression is determined using a z score test (Zar, Biostatistical Analysis, Prentice Hall, Inc., USA, "Differences between Proportions," pp 296-298 (1974)).

Using this approach, a number of polynucleotide sequences were identified as being differentially expressed between, for example, cells derived from high metastatic potential cancer tissue and low metastatic cancer cells, and between cells derived from metastatic cancer tissue and normal tissue. Evaluation of the levels of expression of the genes corresponding to these sequences can be valuable in diagnosis, prognosis, and/or treatment (*e.g.*, to facilitate rationale design of therapy, monitoring during and after therapy, *etc.*). Moreover, the genes corresponding to differentially expressed sequences described herein can be therapeutic targets due to their involvement in regulation (*e.g.*, inhibition or promotion) of development of, for example, the metastatic phenotype. For example, sequences that correspond to genes that are increased in expression in high metastatic potential cells relative to normal or non-metastatic tumor cells may encode genes or regulatory sequences involved in processes such as angiogenesis, differentiation, cell replication, and metastasis.

Detection of the relative expression levels of differentially expressed polynucleotides described herein can provide valuable information to guide the clinician in the choice of therapy. For example, a patient sample exhibiting an expression level of one or more of these polynucleotides that corresponds to a gene that is increased in expression in metastatic or high metastatic potential cells may warrant more aggressive treatment for the patient. In contrast, detection of expression levels of a polynucleotide sequence that corresponds to expression levels associated with that of low metastatic potential cells may warrant a more positive prognosis than

the gross pathology would suggest.

The differential expression of the polynucleotides described herein can thus be used as, for example, diagnostic markers, prognostic markers, for risk assessment, patient treatment and the like. These polynucleotide sequences can also be used in combination with other known molecular and/or biochemical markers.

The differential expression data for polynucleotides of the invention that have been identified as being differentially expressed across various combinations of the libraries described above is summarized in Table 4 (inserted prior to the claims). Table 4 provides: 1) the Sequence Identification Number ("SEQ ID") assigned to the polynucleotide; 2) the cluster ("CLUST") to which the polynucleotide has been assigned as described above; 3) the library comparisons that resulted in identification of the polynucleotide as being differentially expressed ("PairAB-text"), with shorthand names of the compared libraries provided in parentheses following the library numbers; 4) the number of clones corresponding to the polynucleotide in the first library listed ("A"); 5) the number of clones corresponding to the polynucleotide in the second library listed ("B"); 6) the "RATIO PLUS" where the comparison resulted in a finding that the number of clones in library A is greater than the number of clones in library B; and 7) the "RATIO MINUS" where the comparison resulted in a finding that the number of clones in library B is greater than the number of clones in library A.

Example 4: Differential Expression of a Polynucleotides Associated with Metastatic Potential in Breast Cancer

Differential expression was examined in breast cancer cells having either high metastatic potential or low metastatic potential. A single cluster, Cluster Identification No. 10154, was identified as displaying low expression in the high metastatic potential breast cancer cells (Library 3), and significantly increased expression -- approximately 100-fold higher -- in the low metastatic potential cells (Library 4). Specifically, three clones were identified that were expressed in Library 3, the high metastatic potential breast cancer library, while 317 clones were expressed in Library 4, the low metastatic potential breast cancer library. The two sequences assigned to this particular cluster, SEQ ID NO:315 and SEQ ID NO:316, both displayed this differential expression, suggesting that the two sequences are likely associated with a single transcript.

SEQ ID NO:315 and SEQ ID NO:316 were then used as query sequences to search for homologous sequences in GenBank as described in Examples 1 and 2. SEQ ID NO: 315 displayed identity to the GenBank entry H72034 (SEQ ID NO:317) and SEQ ID NO:316 displayed identity to GenBank entry AA707002 (SEQ ID NO:318). SEQ ID NO:315 displays striking identity to the 3' end of SEQ ID NO:317 (See Figures 1A and 1B), while SEQ ID NO:316 displays striking identity to the 5' end of SEQ ID NO:318 (See Figure 2). Clones of H72034 and AA707002 were ordered

from the I.M.A.G.E. Consortium at the Lawrence Livermore National Laboratories (Livermore, California) for further studies.

Restriction Mapping of Clones H72034 and AA707002

- 5 The newly identified sequences were digested with a number of different restriction endonucleases to construct a restriction map of each of the clones. An appropriate amount of each clone, SEQ ID NO:317 or SEQ ID NO:318, was digested with various enzymes, and the restriction fragments identified as follows:

SEQ ID NO:317

	Enzyme	#Cuts	Positions							
	AluI	5	331	1029	1422	1595	1977			
	BamHI	2	1836	2089						
5	BstEII	1	936							
	BstXI	1	1033							
	HaeIII	12	145	300	453	497	582	780		
			1102	1536	1561	1722	1981	2062		
	HinfI	12	5	154	205	325	397	473	610	820
10	968			1295	1426	2066				
	KpnI	1	1938							
	MspI	6	78	739	1098	2038	2077	2093		
	NcoI	2	2013	2058						
	PstI	1	1501							
15	PvuII	2	331	1422						
	Sau3AI	6	1270	1813	1819	1836	1894	2089		
	SphI	1	1870							
	XhoI	1	1413							

20

SEQ ID NO:318

	Enzyme	#Cuts	Positions							
	AluI	9	19	245	367	553	586	874	904	996
	1214									
25	BamHI	1	407							
	BglI	1	1056							
	BglII	1	475							
	BstEI	1	1108							
	HaeIII	10	153	348	485	867	518	628	780	867
30	915			1016	1312					
	HindIII	2	243	872						
	HinfI	1	1353							
	KpnI	1	132							
	MspI	2	1196	1261						
35	PstI	1	823							
	PvuII	1	996							
	Sau3AI	7	66	407	475	504	750	850	1024	

The restriction maps based on the identified sites can be used to determine the position of each clone relative to the genomic sequences, and to confirm the 5'-3' orientation of the clones.

Amplification and Purification of Transcript

A transcript in this region upregulated in low metastatic cancers which contain sequences from SEQ ID NOS: 315-318 is identified using a technique such as polymerase chain reaction (PCR) amplification. Based on the sequences identified and the original sequences of the cluster, primers can be designed to isolate the full length cDNA from a library constructed from the breast cancer cell line with low metastatic potential.

A cDNA template for use in the amplification reaction is generated from total RNA isolated from the high metastatic breast cell line. RNA is reverse transcribed using oligo-dT primer to generate first strand cDNA. cDNA is synthesized by denaturing 3 µl of total RNA, 2 µl oligo-dT primer at 20 µM, and 5 µl DEPC water for 8 minutes at 65°C followed by reverse transcription at 52°C for 1 hour in a reaction containing the denatured RNA/primer plus 4 µl 1 5X cDNA buffer (GibcoBRL), 1 µl 0.1 M dithiothreitol, 1 µl 40 U/l RNaseOUT (GibcoBRL), 1 µl DEPC water, 2 µl 10 mM dNTP (GibcoBRL), and 1 µl 15 U/l Thermoscript reverse transcriptase (GibcoBRL). The reaction was terminated by a 5-min incubation at 85°C, and the RNA was removed by 1 µl 2 U/l RNase H at 37°C for thirty minutes.

Based on the determined orientation of the clones, primers are designed to amplify a full-length clone corresponding to the differentially expressed transcript in this region. Forward primers that are used to amplify the full-length clone are taken from the 5' end of SEQ ID NO:17 as follows:

F1 5'- TGGGATATAGTCTCGTGGTGCG -3' (SEQ ID NO:319)

F2 5'- TGATTCGATGTCATCAGTCCCG-3' (SEQ ID NO:320)

Primer F1 is taken from residues 51-62 of SEQ ID NO: 317, and primer F2 is taken from residues 212-233 Of SEQ ID NO:17. Both forward primers are near the 5' end of this sequence.

Reverse Primers are designed using sequences complementary to the 3' end of clone 10154-3 as follows:

R1 5'- TGTGTCACAGCCAGACATGAGC (SEQ ID NO:321)

R2 5' – TGCAAACATACACAGGGACCG (SEQ ID NO:322)

Primer R1 is based on residues 573-552 of SEQ ID NO:318, and R2 is based on residues 399-379 of SEQ ID NO:318.

PCR is performed using a 5µl aliquot of the first strand cDNA synthesis reaction, and a primer pair, e.g., F1 and R1, F1 and R2, F2 and R1, or F2 and R2. An open reading frame is amplified using 2 µl of the reverse transcription product as template in a PCR reaction containing 5 µl of 10x PCR buffer (GibcoBRL), 1 µl 50 mM Mg₂SO₄, 1 µl 10 mM dNTP, 1 µl F1 or F2 primer, 1 µl R1 primer, 2.5 U High Fidelity Platinum Taq DNA polymerase (GibcoBRL), and water to 50 µl. The molecule is amplified using 30 rounds of amplification in a thermal cycler at the following temperatures: 1 minute at 95°C; 1 minute at 55°C and 2 minutes at 72°C. The 30 cycles was followed by a 10 minute extension at 72°C.

Following amplification of the sequences, the PCR products are loaded on a 1% TEA gel and subjected to gel purification. One or more bands can be isolated from the gel and the DNA was purified using a QIAquick® Gel Extraction Kit (Qiagen, Valencia, CA). The purified fragment was cloned into a bacterial vector and transformed into the bacterial strain DH5α. Following cloning of the purified fragment(s), the DNA can be isolated and sequenced to confirm that a band corresponds to a transcript from this genetic region.

The reactions are carried out with two different 5' and 3' primers to increase the likelihood that the reaction will yield an amplification product. Other primers may also be designed from the predicted 5' and/or 3' end of the sequence, as will be apparent to one skilled in the art upon reading this disclosure, and thus other primers may be designed from the general region of SEQ ID NOS:317 and 318 that may yield better results than the disclosed primers.

In order to obtain additional sequences 5' to the end of a partial cDNA, 5' rapid amplification of cDNA ends (RACE) can be performed to ensure that the entire transcript has been identified. See *PCR Protocols: A Guide to Methods and Applications*, (1990) Academic Press, Inc. Following isolation of a cDNA using the F1-R1 or F2-R1 primer pairs, additional primers can be designed to perform RACE. The primers can be designed from the sequence of 10154-1 as follows:

5'-TTTAGCAGCACTAATGACTGTGGC-3' (SEQ ID NO:323)
5'-CGCCGTGAATTACTGTGGATGG-3' (SEQ ID NO:324)

The two RACE primers are designed based residues 286-263 and 396-375 of SEQ ID NO:317, respectively.

These sequences can be used to obtain any transcript sequences 5' to the amplification products obtained using the PCR protocol described above.

Northern Analysis

Other techniques can be used for confirming differential expression of the full-length transcript. For example, a Northern Blot can be used to verify differential expression of SEQ ID

NOS:317 and 318 in a breast cancer cells with low metastatic potential compared to breast cancer cells with high metastatic potential. Northern analysis can be accomplished by methods well-known in the art. Briefly, RNA is individually isolated from breast cancer cells having high metastatic potential and breast cancer cells having low metastatic potential, *e.g.*, a product such as RNeasy Mini Kits (Qiagen, CA) or NucleoSpin® RNA II Kit (Clontech, Palo Alto, CA). The isolated RNA samples are For Northern analysis, RNA isolated from the cells was electrophoresed on a denaturing formaldehyde agarose gel and transferred onto a membrane such as a supported nitrocellulose membrane (Schleicher & Schuell).

Rapid-Hyb buffer (Amersham Life Science, Little Chalfont, England) with 5 mg/ml denatured single stranded sperm DNA is pre-warmed to 65°C and the RNA blots are pre-hybridized in the buffer with shaking at 65°C for 30 minutes. Gene-specific DNA probes (50 ng per reaction) labeled with [α -³²P]dCTP (3000Ci/mmol, Amersham Pharmacia Biotech Inc., Piscataway, NJ) (Prime-It RmT Kit, Stratagene, La Jolla, CA) and purified with ProbeQuant™ G-50 Micro Columns (Amersham Pharmacia Biotech Inc.) are added and hybridized to the blots with shaking at 65°C for overnight. The blots are washed in 2x SSC, 0.1%(w/v) SDS at room temperature for 20 minutes, twice in 1x SSC, 0.1%(w/v) SDS at 65°C for 15 minutes, then exposed to Hyperfilms (Amersham Life Science).

Example 6: Identification of Differentially Expressed Genes by Array Analysis with Patient Tissue

Samples

Differentially expressed genes corresponding to the polynucleotides described herein were also identified by microarray hybridization analysis using materials obtained from patient tissue samples. The biological materials used in these experiments are described below.

Source of patient tissue samples

Normal and cancerous tissues were collected from patients using laser capture microdissection (LCM) techniques, which techniques are well known in the art (see, *e.g.*, Ohyama *et al.* (2000) *Biotechniques* 29:530-6; Curran *et al.* (2000) *Mol. Pathol.* 53:64-8; Suarez-Quian *et al.* (1999) *Biotechniques* 26:328-35; Simone *et al.* (1998) *Trends Genet* 14:272-6; Conia *et al.* (1997) *J. Clin. Lab. Anal.* 11:28-38; Emmert-Buck *et al.* (1996) *Science* 274:998-1001). Table 8 (inserted following the last page of the Examples) provides information about each patient from which the samples were isolated, including: the Patient ID and Path ReportID, numbers assigned to the patient and the pathology reports for identification purposes; the anatomical location of the tumor (AnatomicalLoc); The Primary Tumor Size; the Primary Tumor Grade; the Histopathologic Grade; a description of local sites to which the tumor had invaded (Local Invasion); the presence of lymph node metastases (Lymph Node Metastasis); incidence of lymph node metastases (provided as number of lymph nodes positive for metastasis over the number of lymph nodes examined)

(Incidence Lymphnode Metastasis); the Regional Lymphnode Grade; the identification or detection of metastases to sites distant to the tumor and their location (Distant Met & Loc); a description of the distant metastases (Description Distant Met); the grade of distant metastasis (Distant Met Grade); and general comments about the patient or the tumor (Comments). Adenoma was not described in any of the patients. ; adenoma dysplasia (described as hyperplasia by the pathologist) was described in Patient ID No. 695. Extranodal extensions were described in two patients, Patient ID Nos. 784 and 791. Lymphovascular invasion was described in seven patients, Patient ID Nos. 128, 278, 517, 534, 784, 786, and 791.. Crohn's-like infiltrates were described in seven patients, Patient ID Nos. 52, 264, 268, 392, 393, 784, and 791.

Source of polynucleotides on arrays

Polynucleotides on arrays

Polynucleotides spotted on the arrays were generated by PCR amplification of clones derived from cDNA libraries. The clones used for amplification were either the clones from which the sequences described herein (SEQ ID NOS:1-316) were derived, or are clones having inserts with significant polynucleotide sequence overlap with the sequences described herein (SEQ ID NO:1-316) as determined by BLAST2 homology searching.

Microarray Design

Each array used in the examples below had an identical spatial layout and control spot set. Each microarray was divided into two areas, each area having an array with, on each half, twelve groupings of 32 x 12 spots for a total of about 9,216 spots on each array. The two areas are spotted identically which provide for at least two duplicates of each clone per array. Spotting was accomplished using PCR amplified products from 0.5kb to 2.0 kb and spotted using a Molecular Dynamics Gen III spotter according to the manufacturer's recommendations. The first row of each of the 24 regions on the array had about 32 control spots, including 4 negative control spots and 8 test polynucleotides.

The test polynucleotides were spiked into each sample before the labeling reaction with a range of concentrations from 2-600 pg/slide and ratios of 1:1. For each array design, two slides were hybridized with the test samples reverse-labeled in the labeling reaction. This provided for about 4 duplicate measurements for each clone, two of one color and two of the other, for each sample.

Microarray analysis

cDNA probes were prepared from total RNA isolated from the patient cells described in above (Table 8). Since LCM provides for the isolation of specific cell types to provide a substantially homogenous cell sample, this provided for a similarly pure RNA sample.

Total RNA was first reverse transcribed into cDNA using a primer containing a T7 RNA polymerase promoter, followed by second strand DNA synthesis. cDNA was then transcribed *in*

vitro to produce antisense RNA using the T7 promoter-mediated expression (see, e.g., Luo *et al.* (1999) *Nature Med* 5:117-122), and the antisense RNA was then converted into cDNA. The second set of cDNAs were again transcribed *in vitro*, using the T7 promoter, to provide antisense RNA. Optionally, the RNA was again converted into cDNA, allowing for up to a third round of
5 T7-mediated amplification to produce more antisense RNA. Thus the procedure provided for two or three rounds of *in vitro* transcription to produce the final RNA used for fluorescent labeling. Fluorescent probes were generated by first adding control RNA to the antisense RNA mix, and producing fluorescently labeled cDNA from the RNA starting material. Fluorescently labeled cDNAs prepared from the tumor RNA sample were compared to fluorescently labeled cDNAs
10 prepared from normal cell RNA sample. For example, the cDNA probes from the normal cells were labeled with Cy3 fluorescent dye (green) and the cDNA probes prepared from the tumor cells were labeled with Cy5 fluorescent dye (red).

The differential expression assay was performed by mixing equal amounts of probes from tumor cells and normal cells of the same patient. The arrays were prehybridized by incubation for
15 about 2 hrs at 60°C in 5X SSC/0.2% SDS/1 mM EDTA, and then washed three times in water and twice in isopropanol. Following prehybridization of the array, the probe mixture was then hybridized to the array under conditions of high stringency (overnight at 42°C in 50% formamide, 5X SSC, and 0.2% SDS. After hybridization, the array was washed at 55°C three times as follows:
20 1) first wash in 1X SSC/0.2% SDS; 2) second wash in 0.1X SSC/0.2% SDS; and 3) third wash in 0.1X SSC.

The arrays were then scanned for green and red fluorescence using a Molecular Dynamics Generation III dual color laser-scanner/detector. The images were processed using BioDiscovery Autogene software, and the data from each scan set normalized to provide for a ratio of expression relative to normal. Data from the microarray experiments was analyzed according to the algorithms
25 described in U.S. application serial no. 60/252,358, filed November 20, 2000, by E.J. Moler, M.A. Boyle, and F.M. Randazzo, and entitled "Precision and accuracy in cDNA microarray data," which application is specifically incorporated herein by reference.

The experiment was repeated, this time labeling the two probes with the opposite color in order to perform the assay in both "color directions." Each experiment was sometimes repeated
30 with two more slides (one in each color direction). The level fluorescence for each sequence on the array expressed as a ratio of the geometric mean of 8 replicate spots/genes from the four arrays or 4 replicate spots/gene from 2 arrays or some other permutation. The data were normalized using the spiked positive controls present in each duplicated area, and the precision of this normalization was included in the final determination of the significance of each differential. The fluorescent intensity
35 of each spot was also compared to the negative controls in each duplicated area to determine which spots have detected significant expression levels in each sample.

A statistical analysis of the fluorescent intensities was applied to each set of duplicate spots to assess the precision and significance of each differential measurement, resulting in a p-value testing the null hypothesis that there is no differential in the expression level between the tumor and normal samples of each patient. For initial analysis of the microarrays, the hypothesis was accepted if $p > 10^{-3}$, and the differential ratio was set to 1.000 for those spots. All other spots have a significant difference in expression between the tumor and normal sample. If the tumor sample has detectable expression and the normal does not, the ratio is truncated at 1000 since the value for expression in the normal sample would be zero, and the ratio would not be a mathematically useful value (e.g., infinity). If the normal sample has detectable expression and the tumor does not, the ratio is truncated to 0.001, since the value for expression in the tumor sample would be zero and the ratio would not be a mathematically useful value. These latter two situations are referred to herein as "on/off." Database tables were populated using a 95% confidence level ($p > 0.05$).

Table 9 below summarize the results of the differential expression analysis. Each table provides: the SEQ ID NO of the polynucleotide corresponding to the polynucleotide on the spot on the array; the Spot ID (an identifier assigned to the spot so as to distinguish it from spots on the same and different arrays), the number of patients for whom there was information obtained from the array (Num Ratios), and the percentage of patients in which expression was detected at greater than or equal to a two-fold increase ($\geq 2x$), greater than or equal to a five-fold increase ($\geq 5x$), or less than or equal to a 1/2 -fold decrease ($\leq \text{half}x$) relative to matched normal control tissue.

In general, a polynucleotide is said to represent a significantly differentially expressed gene between two samples when there is detectable levels of expression in at least one sample and the ratio value is greater than at least about 1.2 fold, preferably greater than at least about 1.5 fold, more preferably greater than at least about 2 fold, where the ratio value is calculated using the method described above.

A differential expression ratio of 1 indicates that the expression level of the gene in the tumor cell was not statistically different from expression of that gene in normal colon cells of the same patient. A differential expression ratio significantly greater than 1 in cancerous colon cells relative to normal colon cells indicates that the gene is increased in expression in cancerous cells relative to normal cells, indicating that the gene plays a role in the development of the cancerous phenotype, and may be involved in promoting metastasis of the cell. Detection of gene products from such genes can provide an indicator that the cell is cancerous, and may provide a therapeutic and/or diagnostic target.

Likewise, a differential expression ratio significantly less than 1 in cancerous colon cells relative to normal colon cells indicates that, for example, the gene is involved in suppression of the cancerous phenotype. Increasing activity of the gene product encoded by such a gene, or replacing such activity, can provide the basis for chemotherapy. Such gene can also serve as markers of

cancerous cells, e.g., the absence or decreased presence of the gene product in a colon cell relative to a normal colon cell indicates that the cell may be cancerous.

Table 9.

SEQ ID NO:	SpotID	Num Ratios	$\geq 2x$	$\geq 5x$	$\leq \text{halfx}$
8	579	33	87.88	39.39	3.03
12	22300	33	33.33	18.18	6.06
26	21886	33	33.33	0.00	3.03
64	9487	33	33.33	12.12	3.03
248	28179	28	32.14	0.00	0.00
253	28179	28	32.14	0.00	0.00
272	28179	28	32.14	0.00	0.00
292	9111	33	33.33	18.18	3.03
295	19980	33	33.33	6.06	0.00
309	23993	33	42.42	3.03	3.03

5

Deposit Information. The following materials were deposited with the American Type Culture Collection (CMCC = Chiron Master Culture Collection).

Table 5. Cell Lines Deposited with ATCC

Cell Line	Deposit Date	ATCC Accession No.	CMCC Accession No.
KM12L4-A	March 19, 1998	CRL-12496	11606
Km12C	May 15, 1998	CRL-12533	11611
MDA-MB-231	May 15, 1998	CRL-12532	10583
MCF-7	October 9, 1998	CRL-12584	10377

10

In addition, pools of selected clones, as well as libraries containing specific clones, were assigned an "ES" number (internal reference) and deposited with the ATCC. Table 6 below provides the ATCC Accession Nos. of the ES deposits, all of which were deposited on or before May 13, 1999. The names of the clones contained within each of these deposits are provided in the Table 7 (inserted before the claims).

15

Table 6: Pools of Clones and Libraries Deposited with ATCC on or before March 28, 2000

Cell Line	CMCC	ATCC
ES75	5140	PTA-1102
ES76	5141	PTA-1103
ES77	5142	PTA-1104
ES78	5143	PTA-1105
ES79	5144	PTA-1106
ES80	5145	PTA-1107
ES81	5146	PTA-1108
ES82	5147	PTA-1109
ES83	5148	PTA-1110
ES84	5149	PTA-1111

The deposits described herein are provided merely as convenience to those of skill in the art, and is not an admission that a deposit is required under 35 U.S.C. §112. The sequence of the polynucleotides contained within the deposited material, as well as the amino acid sequence of the polypeptides encoded thereby, are incorporated herein by reference and are controlling in the event of any conflict with the written description of sequences herein. A license may be required to make, use, or sell the deposited material, and no such license is granted hereby.

Retrieval of Individual Clones from Deposit of Pooled Clones. Where the ATCC deposit is composed of a pool of cDNA clones or a library of cDNA clones, the deposit was prepared by first transfecting each of the clones into separate bacterial cells. The clones in the pool or library were then deposited as a pool of equal mixtures in the composite deposit. Particular clones can be obtained from the composite deposit using methods well known in the art. For example, a bacterial cell containing a particular clone can be identified by isolating single colonies, and identifying colonies containing the specific clone through standard colony hybridization techniques, using an oligonucleotide probe or probes designed to specifically hybridize to a sequence of the clone insert (*e.g.*, a probe based upon unmasked sequence of the encoded polynucleotide having the indicated SEQ ID NO). The probe should be designed to have a T_m of approximately 80°C (assuming 2°C for each A or T and 4°C for each G or C). Positive colonies can then be picked, grown in culture, and the recombinant clone isolated. Alternatively, probes designed in this manner can be used to PCR to isolate a nucleic acid molecule from the pooled clones according to methods well known in the art, *e.g.*, by purifying the cDNA from the deposited culture pool, and using the probes in PCR reactions to produce an amplified product having the corresponding desired polynucleotide sequence.

Those skilled in the art will recognize, or be able to ascertain, using not more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such specific embodiments and equivalents are intended to be encompassed by the following claims.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. The entire contents of the priority documents, as recited in the Application Data Sheet accompanying this application, are also incorporated by reference herein. The citation of any publication is for its disclosure prior to the filing date and should not be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it is readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

Table 1

SEQ ID	CLUSTER	SEQ NAME	ORIENT	CLONE ID	LIBRARY
1	819545	RTA22200265F.k.06.1.P.Seq	F	M00064554D:A03	CH22PRC
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3	818497	RTA22200252F.a.13.1.P.Seq	F	M00063514C:D03	CH21PRN
4	819498	RTA22200252F.n.05.1.P.Seq	F	M00063638C:G12	CH21PRN
5	455465	RTA22200264F.e.16.1.P.Seq	F	M00064454A:H10	CH22PRC
6	819069	RTA22200255F.f.01.1.P.Seq	F	M00063940D:F09	CH21PRN
7	672003	RTA22200265F.b.09.1.P.Seq	F	M00064517C:F11	CH22PRC
8	728115	RTA22200253F.o.24.1.P.Seq	F	M00063838B:G08	CH21PRN
9	372700	RTA22200260F.b.20.1.P.Seq	F	M00063580C:A06	CH22PRC
10	818056	RTA22200266F.c.13.1.P.Seq	F	M00064593D:C01	CH22PRC
11	818497	RTA22200255F.a.17.1.P.Seq	F	M00063920D:H02	CH21PRN
12	729832	RTA22200267F.l.21.1.P.Seq	F	M00064714A:G03	CH22PRC
13	505514	RTA22200251F.b.21.1.P.Seq	F	M00063158A:A01	CH21PRN
14	376488	RTA22200254F.c.05.1.P.Seq	F	M00063852B:D08	CH21PRN
15	376488	RTA22200260F.b.09.1.P.Seq	F	M00063578C:A06	CH22PRC
16	748572	RTA22200254F.c.07.1.P.Seq	F	M00063852D:F07	CH21PRN
17	549934	RTA22200253F.k.18.1.P.Seq	F	M00063801B:D04	CH21PRN
18	819069	RTA22200255F.e.24.1.P.Seq	F	M00063940D:F09	CH21PRN
19	817618	RTA22200253F.n.16.1.P.Seq	F	M00063828D:E05	CH21PRN
20	124396	RTA22200263F.a.11.2.P.Seq	F	M00064375B:G07	CH22PRC
21	404375	RTA22200260F.m.08.1.P.Seq	F	M00063967D:G02	CH22PRC
22	391820	RTA22200261F.f.02.1.P.Seq	F	M00064000B:C03	CH22PRC
23	672003	RTA22200267F.i.06.1.P.Seq	F	M00064693D:F08	CH22PRC
24	830620	RTA22200263F.n.09.1.P.Seq	F	M00064424B:C12	CH22PRC
25	450399	RTA22200251F.f.23.1.P.Seq	F	M00063467D:H07	CH21PRN
26	450982	RTA22200261F.n.18.1.P.Seq	F	M00064307B:G02	CH22PRC
27	819894	RTA22200264F.h.18.1.P.Seq	F	M00064467B:D06	CH22PRC
28	379302	RTA22200257F.j.02.3.P.Seq	F	M00064178C:C04	CH21PRN
29	379746	RTA22200256F.e.16.1.P.Seq	F	M00064086C:E01	CH21PRN
30	124863	RTA22200265F.m.06.1.P.Seq	F	M00064564A:C02	CH22PRC
31	379154	RTA22200257F.c.11.1.P.Seq	F	M00064151B:C07	CH21PRN
32	830620	RTA22200262F.l.23.1.P.Seq	F	M00064358C:D09	CH22PRC
33	389409	RTA22200266F.l.24.1.P.Seq	F	M00064631A:C07	CH22PRC
34	397284	RTA22200262F.i.22.1.P.Seq	F	M00064346C:B09	CH22PRC
35	819440	RTA22200264F.e.19.1.P.Seq	F	M00064454C:B06	CH22PRC
36	389409	RTA22200266F.m.01.1.P.Seq	F	M00064631A:C07	CH22PRC
37	518848	RTA22200265F.n.15.1.P.Seq	F	M00064571C:C04	CH22PRC
38	830620	RTA22200263F.a.21.1.P.Seq	F	M00064376A:A05	CH22PRC
39	379154	RTA22200256F.f.20.1.P.Seq	F	M00064090D:D09	CH21PRN
40	818544	RTA22200256F.h.04.1.P.Seq	F	M00064105B:A03	CH21PRN
41	817375	RTA22200251F.a.15.1.P.Seq	F	M00063152C:B07	CH21PRN

Table 1

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44	377696	RTA22200256F.d.21.1.P.Seq	F	M00064082D:D10	CH21PRN
45	375596	RTA22200261F.h.10.1.P.Seq	F	M00064009A:C01	CH22PRC
46	817689	RTA22200263F.h.05.1.P.Seq	F	M00064399A:E01	CH22PRC
47	831867	RTA22200262F.i.15.2.P.Seq	F	M00064345A:A03	CH22PRC
48	830085	RTA22200261F.k.14.1.P.Seq	F	M00064293D:B12	CH22PRC
49	389627	RTA22200264F.c.10.1.P.Seq	F	M00064447B:C06	CH22PRC
50	397284	RTA22200259F.k.09.1.P.Seq	F	M00063555B:D01	CH22PRC
51	380063	RTA22200261F.j.02.1.P.Seq	F	M00064014D:H05	CH22PRC
52	830931	RTA22200266F.m.23.1.P.Seq	F	M00064633C:A03	CH22PRC
53	819321	RTA22200257F.l.03.3.P.Seq	F	M00064194C:D02	CH21PRN
54	475587	RTA22200261F.c.01.1.P.Seq	F	M00063990A:D05	CH22PRC
55	819046	RTA22200255F.a.18.1.P.Seq	F	M00063920D:H05	CH21PRN
56	817477	RTA22200253F.g.21.1.P.Seq	F	M00063784A:H12	CH21PRN
57	475587	RTA22200261F.b.24.1.P.Seq	F	M00063990A:D05	CH22PRC
58	728115	RTA22200253F.p.01.1.P.Seq	F	M00063838B:G08	CH21PRN
59	389627	RTA22200260F.i.24.1.P.Seq	F	M00063957A:E02	CH22PRC
60	403453	RTA22200256F.i.24.1.P.Seq	F	M00064113B:C04	CH21PRN
61	508525	RTA22200255F.d.10.1.P.Seq	F	M00063931B:F07	CH21PRN
62	819525	RTA22200261F.n.20.1.P.Seq	F	M00064307C:G03	CH22PRC
63	817618	RTA22200255F.i.03.1.P.Seq	F	M00064025D:H12	CH21PRN
64	819403	RTA22200254F.h.14.1.P.Seq	F	M00063888D:D05	CH21PRN
65	553242	RTA22200254F.g.20.1.P.Seq	F	M00063886A:B06	CH21PRN
66	817417	RTA22200255F.a.10.1.P.Seq	F	M00063919C:E07	CH21PRN
67	817618	RTA22200252F.f.13.1.P.Seq	F	M00063604A:B11	CH21PRN
68	611440	RTA22200262F.e.04.2.P.Seq	F	M00064328B:H09	CH22PRC
69	817375	RTA22200260F.m.06.1.P.Seq	F	M00063967C:A12	CH22PRC
70	213577	RTA22200255F.i.23.1.P.Seq	F	M00064033C:C11	CH21PRN
71	820061	RTA22200265F.p.10.1.P.Seq	F	M00064579D:E11	CH22PRC
72	455264	RTA22200259F.m.06.1.P.Seq	F	M00063559D:G03	CH22PRC
73	455264	RTA22200255F.o.23.1.P.Seq	F	M00064059A:C11	CH21PRN
74	380331	RTA22200255F.b.19.1.P.Seq	F	M00063926A:H04	CH21PRN
75	380331	RTA22200252F.b.19.1.P.Seq	F	M00063518D:A01	CH21PRN
76	817455	RTA22200267F.o.01.1.P.Seq	F	M00064723D:H03	CH22PRC
77	423967	RTA22200252F.a.20.1.P.Seq	F	M00063515B:H02	CH21PRN
78	220584	RTA22200261F.m.14.1.P.Seq	F	M00064302A:D10	CH22PRC
79	817688	RTA22200251F.e.20.1.P.Seq	F	M00063462D:D07	CH21PRN
80	549934	RTA22200253F.n.10.1.P.Seq	F	M00063826A:D03	CH21PRN
81	819149	RTA22200255F.e.16.1.P.Seq	F	M00063938B:H07	CH21PRN
82	817455	RTA22200267F.n.24.1.P.Seq	F	M00064723D:H03	CH22PRC

Table 1

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85	194490	RTA22200264F.l.07.1.P.Seq	F	M00064481C:F03	CH22PRC
86	819460	RTA22200257F.m.15.3.P.Seq	F	M00064200D:E08	CH21PRN
87	819018	RTA22200257F.p.01.3.P.Seq	F	M00064212D:E04	CH21PRN
88	830620	RTA22200259F.p.24.1.P.Seq	F	M00063571B:G03	CH22PRC
89	141079	RTA22200262F.k.19.1.P.Seq	F	M00064354A:A10	CH22PRC
90	376588	RTA22200256F.e.04.1.P.Seq	F	M00064083D:E05	CH21PRN
91	380604	RTA22200264F.g.05.1.P.Seq	F	M00064460C:B01	CH22PRC
92	413138	RTA22200260F.b.05.1.P.Seq	F	M00063577C:C02	CH22PRC
93	818544	RTA22200265F.e.12.1.P.Seq	F	M00064527A:H07	CH22PRC
94	647435	RTA22200257F.h.08.1.P.Seq	F	M00064172C:A02	CH21PRN
95	551785	RTA22200266F.c.09.1.P.Seq	F	M00064593A:A05	CH22PRC
96	17092	RTA22200261F.f.17.1.P.Seq	F	M00064002C:F06	CH22PRC
97	818326	RTA22200251F.i.06.1.P.Seq	F	M00063478C:D01	CH21PRN
98	377944	RTA22200262F.e.03.2.P.Seq	F	M00064328B:H04	CH22PRC
99	745559	RTA22200262F.m.04.1.P.Seq	F	M00064359B:H12	CH22PRC
100	818326	RTA22200265F.d.08.1.P.Seq	F	M00064524A:A09	CH22PRC
101	379879	RTA22200264F.b.23.1.P.Seq	F	M00064446A:D11	CH22PRC
102	819640	RTA22200257F.f.24.1.P.Seq	F	M00064165A:B12	CH21PRN
103	818326	RTA22200265F.a.14.1.P.Seq	F	M00064514D:F11	CH22PRC
104	243524	RTA22200265F.g.04.1.P.Seq	F	M00064532D:G06	CH22PRC
105	43995	RTA22200261F.l.02.1.P.Seq	F	M00064294D:F01	CH22PRC
106	597854	RTA22200262F.g.06.2.P.Seq	F	M00064337D:F01	CH22PRC
107	268290	RTA22200260F.p.14.1.P.Seq	F	M00063981D:A06	CH22PRC
108	818043	RTA22200256F.p.10.2.P.Seq	F	M00064138A:F11	CH21PRN
109	830930	RTA22200267F.b.03.1.P.Seq	F	M00064652B:D09	CH22PRC
110	389627	RTA22200260F.j.01.1.P.Seq	F	M00063957A:E02	CH22PRC
111	378730	RTA22200260F.i.07.1.P.Seq	F	M00063955C:F07	CH22PRC
112	819037	RTA22200260F.n.09.1.P.Seq	F	M00063972C:E10	CH22PRC
113	830397	RTA22200261F.g.14.1.P.Seq	F	M00064005D:A08	CH22PRC
114	450247	RTA22200261F.e.10.1.P.Seq	F	M00063998C:E09	CH22PRC
115	819273	RTA22200252F.b.09.1.P.Seq	F	M00063517A:A04	CH21PRN
116	587779	RTA22200257F.i.11.3.P.Seq	F	M00064175B:B09	CH21PRN
117	818639	RTA22200256F.j.09.1.P.Seq	F	M00064115B:E12	CH21PRN
118	615617	RTA22200261F.o.13.1.P.Seq	F	M00064309C:H09	CH22PRC
119	79309	RTA22200257F.j.13.3.P.Seq	F	M00064180A:G03	CH21PRN
120	748994	RTA22200261F.o.20.1.P.Seq	F	M00064310C:A10	CH22PRC
121	818682	RTA22200258F.h.07.1.P.Seq	F	M00064271B:D03	CH21PRN
122	373061	RTA22200253F.j.09.1.P.Seq	F	M00063795C:D09	CH21PRN
123	484413	RTA22200253F.g.09.1.P.Seq	F	M00063781B:B10	CH21PRN

Table 1

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126	170313	RTA22200255F.g.20.1.P.Seq	F	M00063949D:A05	CH21PRN
127	818682	RTA22200253F.p.14.1.P.Seq	F	M00063841A:B09	CH21PRN
128	377188	RTA22200255F.l.06.1.P.Seq	F	M00064043D:C09	CH21PRN
129	518848	RTA22200257F.j.22.3.P.Seq	F	M00064186C:B03	CH21PRN
130	45592	RTA22200259F.l.08.1.P.Seq	F	M00063557D:C07	CH22PRC
131	819273	RTA22200255F.n.19.1.P.Seq	F	M00064053C:G04	CH21PRN
132	397284	RTA22200251F.a.06.1.P.Seq	F	M00063151D:B10	CH21PRN
133	818326	RTA22200258F.e.14.1.P.Seq	F	M00064260C:E05	CH21PRN
134	819037	RTA22200251F.c.15.1.P.Seq	F	M00063452A:F08	CH21PRN
135	817417	RTA22200253F.m.14.1.P.Seq	F	M00063818C:A09	CH21PRN
136	819640	RTA22200254F.i.11.1.P.Seq	F	M00063891A:F11	CH21PRN
137	818771	RTA22200254F.i.19.1.P.Seq	F	M00063892B:G02	CH21PRN
138	389627	RTA22200254F.k.10.1.P.Seq	F	M00063898A:A10	CH21PRN
139	379067	RTA22200260F.e.20.1.P.Seq	F	M00063593A:D03	CH22PRC
140	818544	RTA22200251F.f.02.1.P.Seq	F	M00063463D:B05	CH21PRN
141	819440	RTA22200251F.j.22.1.P.Seq	F	M00063485A:E05	CH21PRN
142	817417	RTA22200251F.k.10.1.P.Seq	F	M00063487C:C02	CH21PRN
143	385307	RTA22200262F.k.11.1.P.Seq	F	M00064352C:H01	CH22PRC
144	611440	RTA22200263F.d.24.2.P.Seq	F	M00064386B:C02	CH22PRC
145	376056	RTA22200259F.e.16.1.P.Seq	F	M00063538D:B01	CH22PRC
146	611440	RTA22200263F.d.24.1.P.Seq	F	M00064386B:C02	CH22PRC
147	820061	RTA22200264F.f.09.1.P.Seq	F	M00064457D:C09	CH22PRC
148	617825	RTA22200264F.p.06.1.P.Seq	F	M00064508A:B09	CH22PRC
149	819440	RTA22200257F.h.17.1.P.Seq	F	M00064173B:E01	CH21PRN
150	819145	RTA22200266F.m.08.1.P.Seq	F	M00064631C:H11	CH22PRC
151	817653	RTA22200265F.p.07.1.P.Seq	F	M00064579A:C06	CH22PRC
152	611440	RTA22200263F.e.01.1.P.Seq	F	M00064386B:C02	CH22PRC
153	375958	RTA22200264F.j.22.1.P.Seq	F	M00064476D:C04	CH22PRC
154	611440	RTA22200257F.a.20.1.P.Seq	F	M00064144D:A07	CH21PRN
155	831049	RTA22200266F.o.13.1.P.Seq	F	M00064637B:F03	CH22PRC
156	818162	RTA22200266F.g.18.1.P.Seq	F	M00064610D:H01	CH22PRC
157	553200	RTA22200263F.p.02.1.P.Seq	F	M00064429D:B07	CH22PRC
158	139677	RTA22200254F.o.07.1.P.Seq	F	M00063910D:A12	CH21PRN
159	139677	RTA22200252F.c.11.1.P.Seq	F	M00063520D:E11	CH21PRN
160	397284	RTA22200262F.i.22.2.P.Seq	F	M00064346C:B09	CH22PRC
161	385810	RTA22200256F.m.04.2.P.Seq	F	M00064126C:F12	CH21PRN
162	404624	RTA22200261F.e.07.1.P.Seq	F	M00063997C:B12	CH22PRC
163	375958	RTA22200262F.b.14.2.P.Seq	F	M00064322C:A10	CH22PRC
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Table 1

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167	36113	RTA22200265F.e.06.1.P.Seq	F	M00064526D:F05	CH22PRC
168	831812	RTA22200263F.f.05.1.P.Seq	F	M00064390A:C05	CH22PRC
169	817653	RTA22200252F.g.23.1.P.Seq	F	M00063610D:C11	CH21PRN
170	397284	RTA22200252F.m.15.1.P.Seq	F	M00063636A:E01	CH21PRN
171	817979	RTA22200253F.p.15.1.P.Seq	F	M00063841A:E08	CH21PRN
172	817653	RTA22200255F.m.18.1.P.Seq	F	M00064048C:G12	CH21PRN
173	611440	RTA22200253F.f.03.1.P.Seq	F	M00063774A:D09	CH21PRN
174	386014	RTA22200261F.f.06.1.P.Seq	F	M00064001A:B03	CH22PRC
175	549981	RTA22200255F.b.10.1.P.Seq	F	M00063925B:F04	CH21PRN
176	193373	RTA22200255F.l.21.1.P.Seq	F	M00064046A:G02	CH21PRN
177	400619	RTA22200255F.g.14.1.P.Seq	F	M00063947D:D01	CH21PRN
178	831149	RTA22200261F.o.21.1.P.Seq	F	M00064310D:F03	CH22PRC
179	36113	RTA22200255F.d.16.1.P.Seq	F	M00063932D:G08	CH21PRN
180	817503	RTA22200253F.l.16.1.P.Seq	F	M00063805D:E05	CH21PRN
181	376588	RTA22200260F.i.11.1.P.Seq	F	M00063955D:F05	CH22PRC
182	141079	RTA22200252F.f.23.1.P.Seq	F	M00063606C:B04	CH21PRN
183	818063	RTA22200253F.p.04.1.P.Seq	F	M00063839A:F01	CH21PRN
184	455264	RTA22200253F.n.14.1.P.Seq	F	M00063828A:H12	CH21PRN
185	189234	RTA22200251F.f.17.1.P.Seq	F	M00063466C:C11	CH21PRN
186	295694	RTA22200265F.j.05.1.P.Seq	F	M00064550A:A07	CH22PRC
187	648679	RTA22200260F.f.06.1.P.Seq	F	M00063594B:H07	CH22PRC
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192	403453	RTA22200266F.e.10.1.P.Seq	F	M00064601D:B05	CH22PRC
193	730318	RTA22200264F.c.09.1.P.Seq	F	M00064447B:A07	CH22PRC
194	44183	RTA22200271F.a.01.1.P.Seq	F	M00021929A:D03	CH03MAH
195	373928	RTA22200255F.d.22.1.P.Seq	F	M00063934B:E04	CH21PRN
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197	403173	RTA22200253F.a.21.1.P.Seq	F	M00063685A:C02	CH21PRN
198	372700	RTA22200253F.c.06.1.P.Seq	F	M00063689D:E12	CH21PRN
199	374343	RTA22200261F.h.04.1.P.Seq	F	M00064008A:B01	CH22PRC
200	597854	RTA22200255F.j.03.1.P.Seq	F	M00064033D:B01	CH21PRN
201	817417	RTA22200255F.a.23.1.P.Seq	F	M00063922B:A12	CH21PRN
202	818497	RTA22200257F.k.05.3.P.Seq	F	M00064188B:G08	CH21PRN
203	377696	RTA22200255F.f.15.1.P.Seq	F	M00063943B:G12	CH21PRN
204	379105	RTA22200252F.n.19.1.P.Seq	F	M00063642B:A08	CH21PRN
205	831188	RTA22200267F.o.02.1.P.Seq	F	M00064723D:H11	CH22PRC

Table 1

SEQ ID	CLUSTER	SEQ NAME	ORIENT	CLONE ID	LIBRARY
206	376056	RTA22200253F.m.09.1.P.Seq	F	M00063810C:E03	CH21PRN-
207	124863	RTA22200255F.n.15.1.P.Seq	F	M00064053B:D09	CH21PRN
208	376056	RTA22200254F.i.03.1.P.Seq	F	M00063890A:F11	CH21PRN
209	831812	RTA22200266F.j.10.1.P.Seq	F	M00064620C:D01	CH22PRC
210	141079	RTA22200260F.i.14.1.P.Seq	F	M00063956A:F05	CH22PRC
211	19148	RTA22200265F.o.18.1.P.Seq	F	M00064577C:B12	CH22PRC
212	124396	RTA22200252F.a.14.1.P.Seq	F	M00063514C:E08	CH21PRN
213	831026	RTA22200265F.c.03.1.P.Seq	F	M00064520A:F08	CH22PRC
214	819037	RTA22200263F.i.23.1.P.Seq	F	M00064405B:C04	CH22PRC
215	380207	RTA22200263F.i.19.1.P.Seq	F	M00064404C:G05	CH22PRC
216	819460	RTA22200255F.c.13.1.P.Seq	F	M00063928A:G09	CH21PRN
217	379067	RTA22200253F.g.23.1.P.Seq	F	M00063784C:E10	CH21PRN
218	403173	RTA22200252F.p.23.1.P.Seq	F	M00063682A:C04	CH21PRN
219	3856	RTA22200269F.a.05.1.P.Seq	F	M00003773D:H02	CH01COH
220	378551	RTA22200263F.d.17.1.P.Seq	F	M00064385D:C11	CH22PRC
221	456089	RTA22200272F.a.09.1.P.Seq	F	M00043134A:A05	CH19COP
222	549981	RTA22200267F.a.22.1.P.Seq	F	M00064650B:B07	CH22PRC
223	378551	RTA22200265F.m.21.1.P.Seq	F	M00064568A:H06	CH22PRC
224	819201	RTA22200256F.n.23.2.P.Seq	F	M00064132B:B07	CH21PRN
225	374826	RTA22200251F.c.20.1.P.Seq	F	M00063453B:F08	CH21PRN
226	389409	RTA22200253F.l.23.1.P.Seq	F	M00063807A:D12	CH21PRN
227	819149	RTA22200260F.a.17.1.P.Seq	F	M00063575B:G02	CH22PRC
228	389409	RTA22200255F.e.18.1.P.Seq	F	M00063939C:D06	CH21PRN
229	818165	RTA22200254F.h.15.1.P.Seq	F	M00063888D:F02	CH21PRN
230	817757	RTA22200252F.i.15.1.P.Seq	F	M00063617D:F09	CH21PRN
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232	385615	RTA22200265F.b.08.1.P.Seq	F	M00064517B:F10	CH22PRC
233	819102	RTA22200258F.h.19.1.P.Seq	F	M00064272C:G01	CH21PRN
234	817757	RTA22200255F.o.16.1.P.Seq	F	M00064057C:H10	CH21PRN
235	385615	RTA22200265F.b.07.1.P.Seq	F	M00064517B:F04	CH22PRC
236	385615	RTA22200253F.l.06.1.P.Seq	F	M00063804C:A11	CH21PRN
237	827355	RTA22200266F.n.23.1.P.Seq	F	M00064636B:A04	CH22PRC
238	817629	RTA22200259F.a.13.1.P.Seq	F	M00063165A:C09	CH22PRC
239	817514	RTA22200260F.h.02.1.P.Seq	F	M00063600C:C09	CH22PRC
240	817514	RTA22200252F.p.21.1.P.Seq	F	M00063681B:C02	CH21PRN
241	680563	RTA22200265F.f.13.1.P.Seq	F	M00064530B:H02	CH22PRC
242	827355	RTA22200255F.e.20.1.P.Seq	F	M00063939C:H01	CH21PRN
243	377286	RTA22200254F.a.04.1.P.Seq	F	M00063843B:D07	CH21PRN
244	680563	RTA22200258F.g.18.1.P.Seq	F	M00064268D:G03	CH21PRN
245	819156	RTA22200255F.h.06.1.P.Seq	F	M00064021D:H01	CH21PRN
246	220584	RTA22200261F.f.22.1.P.Seq	F	M00064003B:C10	CH22PRC

Table 1

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249	817508	RTA22200257F.h.01.1.P.Seq	F	M00064171D:E05	CH21PRN
250	817690	RTA22200257F.e.05.1.P.Seq	F	M00064159A:H03	CH21PRN
251	819156	RTA22200256F.h.13.1.P.Seq	F	M00064106C:G03	CH21PRN
252	830904	RTA22200266F.j.12.1.P.Seq	F	M00064620D:G05	CH22PRC
253	819498	RTA22200253F.b.04.1.P.Seq	F	M00063686B:E07	CH21PRN
254	817508	RTA22200257F.g.24.1.P.Seq	F	M00064171D:E05	CH21PRN
255	817508	RTA22200252F.a.19.1.P.Seq	F	M00063515B:F06	CH21PRN
256	831160	RTA22200267F.h.01.1.P.Seq	F	M00064690A:C04	CH22PRC
257	817762	RTA22200252F.k.13.1.P.Seq	F	M00063627C:F06	CH21PRN
258	377286	RTA22200266F.k.07.1.P.Seq	F	M00064624C:B03	CH22PRC
259	831160	RTA22200267F.g.24.1.P.Seq	F	M00064690A:C04	CH22PRC
260	819994	RTA22200256F.k.11.1.P.Seq	F	M00064119C:D12	CH21PRN
261	819994	RTA22200256F.k.09.1.P.Seq	F	M00064119B:H10	CH21PRN
262	373298	RTA22200259F.c.19.1.P.Seq	F	M00063533A:C12	CH22PRC
263	819894	RTA22200256F.m.03.2.P.Seq	F	M00064126C:C02	CH21PRN
264	372718	RTA22200260F.b.22.1.P.Seq	F	M00063580D:B06	CH22PRC
265	827355	RTA22200262F.l.20.1.P.Seq	F	M00064358A:G03	CH22PRC
266	819894	RTA22200255F.d.09.1.P.Seq	F	M00063931B:E10	CH21PRN
267	827355	RTA22200266F.e.07.1.P.Seq	F	M00064601C:G07	CH22PRC
268	372718	RTA22200256F.l.03.1.P.Seq	F	M00064122C:B06	CH21PRN
269	647435	RTA22200251F.b.10.1.P.Seq	F	M00063156D:H10	CH21PRN
270	450262	RTA22200265F.a.10.1.P.Seq	F	M00064514A:G10	CH22PRC
271	484703	RTA22200255F.i.20.1.P.Seq	F	M00064032D:G04	CH21PRN
272	819498	RTA22200256F.f.12.1.P.Seq	F	M00064089B:F09	CH21PRN
273	406043	RTA22200263F.i.12.1.P.Seq	F	M00064404A:B05	CH22PRC
274	817500	RTA22200255F.f.24.1.P.Seq	F	M00063945A:C03	CH21PRN
275	818180	RTA22200264F.o.18.1.P.Seq	F	M00064506A:C07	CH22PRC
276	818143	RTA22200251F.a.03.1.P.Seq	F	M00063151A:G06	CH21PRN
277	819756	RTA22200267F.a.18.1.P.Seq	F	M00064649A:E04	CH22PRC
278	406908	RTA22200257F.i.18.3.P.Seq	F	M00064176D:H10	CH21PRN
279	124863	RTA22200256F.o.21.2.P.Seq	F	M00064136C:D12	CH21PRN
280	429009	RTA22200257F.e.24.1.P.Seq	F	M00064161B:G04	CH21PRN
281	402586	RTA22200257F.i.24.3.P.Seq	F	M00064178B:A05	CH21PRN
282	400475	RTA22200254F.i.04.1.P.Seq	F	M00063890A:H04	CH21PRN
283	403453	RTA22200264F.d.12.1.P.Seq	F	M00064450C:E07	CH22PRC
284	383021	RTA22200259F.d.06.1.P.Seq	F	M00063534C:A02	CH22PRC
285	394913	RTA22200254F.p.10.1.P.Seq	F	M00063915C:E01	CH21PRN
286	831361	RTA22200263F.k.19.1.P.Seq	F	M00064414D:D06	CH22PRC
287	646020	RTA22200267F.n.21.1.P.Seq	F	M00064723C:H04	CH22PRC

Table 1

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290	402586	RTA22200257F.j.01.3.P.Seq	F	M00064178B:A05	CH21PRN
291	400475	RTA22200262F.j.21.1.P.Seq	F	M00064349D:H01	CH22PRC
292	818937	RTA22200262F.h.14.2.P.Seq	F	M00064341A:C02	CH22PRC
293	557697	RTA22200261F.j.20.1.P.Seq	F	M00064018C:E07	CH22PRC
294	831361	RTA22200265F.m.24.1.P.Seq	F	M00064569B:A09	CH22PRC
295	194490	RTA22200252F.c.10.1.P.Seq	F	M00063520D:D08	CH21PRN
296	818143	RTA22200254F.b.18.1.P.Seq	F	M00063848C:G11	CH21PRN
297	377286	RTA22200259F.a.10.1.P.Seq	F	M00063163A:G04	CH22PRC
298	831361	RTA22200265F.n.01.1.P.Seq	F	M00064569B:A09	CH22PRC
299	385307	RTA22200255F.p.07.1.P.Seq	F	M00064060B:D03	CH21PRN
300	378447	RTA22200251F.c.01.1.P.Seq	F	M00063158A:E11	CH21PRN
301	378447	RTA22200251F.b.24.1.P.Seq	F	M00063158A:E11	CH21PRN
302	817514	RTA22200260F.m.17.1.P.Seq	F	M00063968D:G08	CH22PRC
303	818942	RTA22200255F.f.03.1.P.Seq	F	M00063941B:C12	CH21PRN
304	818942	RTA22200267F.e.23.1.P.Seq	F	M00064678D:F05	CH22PRC
305	817363	RTA22200266F.f.04.1.P.Seq	F	M00064605C:G05	CH22PRC
306	818942	RTA22200255F.i.02.1.P.Seq	F	M00064025D:E07	CH21PRN
307	818942	RTA22200265F.g.23.1.P.Seq	F	M00064534D:F06	CH22PRC
308	817457	RTA22200267F.e.15.1.P.Seq	F	M00064675C:E09	CH22PRC
309	831968	RTA22200263F.f.23.1.P.Seq	F	M00064393B:H04	CH22PRC
310	530941	RTA22200253F.h.05.1.P.Seq	F	M00063785C:F03	CH21PRN
311	763446	RTA22200257F.j.05.3.P.Seq	F	M00064179A:C04	CH21PRN
312	763446	RTA22200255F.n.21.1.P.Seq	F	M00064053D:F02	CH21PRN
313	819219	RTA22200256F.f.16.1.P.Seq	F	M00064090C:A02	CH21PRN
314	763446	RTA22200258F.b.19.2.P.Seq	F	M00064248A:E02	CH21PRN
315	10154				
316	10154				

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
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19	<NONE>	<NONE>	<NONE>	1077580	hypothetical protein YDR125c - yeast	7
20	<NONE>	<NONE>	<NONE>	4585925	(AC007211) unknown protein	6
21	<NONE>	<NONE>	<NONE>	1085306	EVI1 protein - human	4.3
22	<NONE>	<NONE>	<NONE>	3876587	(Z81521) predicted using Genefinder; cDNA EST yk233g4.5 comes from this gene; cDNA EST yk233g4.3 comes from this gene [Caenorhabditis elegans]	0.85
23	<NONE>	<NONE>	<NONE>	1086591	(U41007) similar to S. cerevisiae nuclear protein SNF2	0.34
24	<NONE>	<NONE>	<NONE>	157272	(L11345) DNA-binding protein [Drosophila melanogaster]	0.29
25	<NONE>	<NONE>	<NONE>	2633160	(Z99108) similar to surface adhesion YfiQ [Bacillus subtilis]	0.19
26	<NONE>	<NONE>	<NONE>	755468	(U19879) transmembrane protein [Xenopus laevis]	0.042
27	<NONE>	<NONE>	<NONE>	4507339	T brachyury (mouse) homolog protein [Homo sapiens]	0.029

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
28	<NONE>	<NONE>	<NONE>	729711	PROTEASE DEGS PRECURSOR 3.4.21.-) hhoB - Escherichia coli >gi 558913 (U15661) HhoB [Escherichia coli] >gi 606174 (U18997) ORF_o355 coli] >gi 1789630 (AE000402) protease [Escherichia coli]	0.004
29	<NONE>	<NONE>	<NONE>	3168911	(AF068718) No definition line found [Caenorhabditis elegans]	8e-013
30	<NONE>	<NONE>	<NONE>	2832777	(AL021086) /prediction=(me thod;; comes from the 5' UTR [Drosophila melanogaster]	3e-040
31	X78712	H.sapiens mRNA for glycerol kinase testis specific 2	2.1	2852449	(D88207) protein kinase [Arabidopsis thaliana] >gi 2947061 (AC002521) putative protein kinase	9.1
32	X60760	L.esculentum TDR8 mRNA	2.1	157272	(L11345) DNA- binding protein [Drosophila melanogaster]	5

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
33	U40853	Oryctolagus cuniculus pulmonary surfactant protein B (SP-B) gene, complete cds	2	< NONE >	< NONE >	< NONE >
34	AF083655	Homo sapiens procollagen C-proteinase enhancer protein (PCOLCE) gene, 5' flanking region and complete cds	2	< NONE >	< NONE >	< NONE >
35	AJ223776	Staphylococcus warneri hld gene	2	< NONE >	< NONE >	< NONE >
36	U40853	Oryctolagus cuniculus pulmonary surfactant protein B (SP-B) gene, complete cds	2	< NONE >	< NONE >	< NONE >
37	X04436	Clostridium tetani gene for tetanus toxin	2	< NONE >	< NONE >	< NONE >
38	Z35787	S.cerevisiae chromosome II reading frame ORF YBL026w	2	157272	(L11345) DNA-binding protein [Drosophila melanogaster]	8.4
39	X78712	H.sapiens mRNA for glycerol kinase testis specific 2	2	2852449	(D88207) protein kinase [Arabidopsis thaliana] > gi 2947061 (AC002521) putative protein kinase	8.2

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
40	Z15056	B.subtilis genes spoVD, murE, mraY, murD	2	477124	P3A2 DNA binding protein homolog EWG - fruit fly (Drosophila melanogaster)	2.8
41	S65623	cAMP-regulated enhancer-binding protein 1 of 3]	2	119266	PROTEIN GRAINY-HEAD (DNA-BINDING PROTEIN ELF-1) (ELEMENT I-BINDING ACTIVITY) regulatory protein elf-1 - fruit fly (Drosophila melanogaster) > gi 7939 emb CAA33692 (X15657) Elf-1 protein (AA 1-1063) [Drosophila melanogaster]	0.55
42	NM_004415.1	Homo sapiens desmoplakin (DPI, DPII) (DSP) mRNA, complete cds	2	2649177	(AE001008) conserved hypothetical protein [Archaeoglobus fulgidus]	0.2
43	AF031552	Vibrio cholerae magnesium transporter (mgtE) gene, partial cds; sensor kinase (vieS), response regulator (vieA), and response regulator (vieB) genes, complete cds; and collagenase (vcc) gene,	2	2088714	(AF003139) strong similarity to NADPH oxidases; partial CDS, the gene begins in the neighboring clone	2e-013

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
		(vcc) gene, partial cds				
44	AF116852.1	Danio rerio dickkopf-1 (dkk1) mRNA, complete cds	2	3800951	(AF100657) No definition line found [Caenorhabditis elegans]	2e-019
45	X82595	P.sativum fuc gene	1.9	<NONE>	<NONE>	<NONE>
46	AF008216	Homo sapiens candidate tumor suppressor pp32r1	1.9	<NONE>	<NONE>	<NONE>
47	AF130672.1	Felis catus clone Fca603 microsatellite sequence	1.9	<NONE>	<NONE>	<NONE>
48	AJ007044	Oryctolagus Cuniculus sod gene	1.9	388055	(L22981) merozoite surface protein-1 [Plasmodium chabaudi]	7.8
49	AC004497	Homo sapiens chromosome 21, P1 clone LBNL#6	1.9	160925	(M94346) A.1.12/9 antigen [Schistosoma mansoni]	7.7

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
50	U30290	Rattus norvegicus galanin receptor GALR1 mRNA, complete cds	1.9	3024079	GALECTIN-4 (LACTOSE-BINDING LECTIN 4) (L-36 LACTOSE BINDING PROTEIN) (L36LBP) > gi 2281707 sapiens] > gi 2623387 (U82953) galectin-4 [Homo sapiens]	4.5
51	Y13234	Chironomus tentans mRNA for chitinase, 1695 bp	1.9	4567068	(AF125568) tumor suppressing STF cDNA 4 [Homo sapiens]	3.4
52	NM_003644.1	Homo sapiens growth arrest-specific 7 (GAS7) mRNA > :: emb AJ224876 HSAJ4876 Homo sapiens mRNA for GAS7 protein	1.9	125560	PROTEIN KINASE C, GAMMA TYPE C (EC 2.7.1.-) gamma - rabbit > gi 165652 (M19338) protein kinase delta [Oryctolagus cuniculus]	0.53
53	AB013448.1	Oryza sativa gene for Pib, complete cds	1.8	<NONE>	<NONE>	<NONE> >
54	D63854	Human cytomegalovirus DNA, replication origin	1.8	<NONE>	<NONE>	<NONE> >
55	AB002340	Human mRNA for KIAA0342 gene, complete cds	1.8	<NONE>	<NONE>	<NONE> >
56	AF017779	Mus musculus vitamin D receptor gene, promoter region	1.8	<NONE>	<NONE>	<NONE> >

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
57	D63854	Human cytomegalovirus DNA, replication origin	1.8	< NONE >	< NONE >	< NONE >
58	M24102	Bovine ADP/ATP translocase T1 mRNA, complete cds.	1.8	< NONE >	< NONE >	< NONE >
59	AC004497	Homo sapiens chromosome 21, P1 clone LBNL#6	1.8	< NONE >	< NONE >	< NONE >
60	M37394	Rat epidermal growth factor receptor mRNA.	1.8	< NONE >	< NONE >	< NONE >
61	AF006304	Saccharomyces cerevisiae protein tyrosine phosphatase (PTP3) gene, complete cds	1.8	< NONE >	< NONE >	< NONE >
62	D13454	Candida albicans CACHS3 gene for chitin synthase III	1.8	< NONE >	< NONE >	< NONE >
63	Y00354	Xenopus laevis gene encoding vitellogenin A2	1.8	1077580	hypothetical protein YDR125c - yeast	7.5
64	U90936	Aspergillus niger px27 gene, promoter region	1.8	4337033	(AF124138) transcriptional activator protein CdaR [Streptomyces coelicolor] transcriptional regulator [Streptomyces coelicolor]	7.3

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
65	D84448	<i>Cavia cobaya</i> mRNA for Na ⁺ , K ⁺ -ATPase beta-3 subunit, complete cds	1.8	4704603	(AF109916) putative dehydrin	7.1
66	AF039948	<i>Xenopus laevis</i> clone H-0 transcription elongation factor S-II (TFIIS) precursor RNA, isoform TFIIS.h, partial cds	1.8	1695839	(U58151) envelope glycoprotein [Human immunodeficiency virus type 1]	5.6
67	M18061	<i>Xenopus laevis</i> vitellogenin gene, complete cds.	1.8	780502	(U18466) AP endonuclease class II [African swine fever virus] >gi 1097525 p rf 2113434ET AP endonuclease:IS OTYPE=class II [African swine fever virus]	3.1
68	U61112	<i>Mus musculus</i> Eya3 homolog mRNA, complete cds	1.8	3043646	(AB011133) KIAA0561 protein [Homo sapiens]	1.9
69	AB018442	<i>Oryza sativa</i> mRNA for phytochrome C, complete cds	1.8	4455041	(AF116463) unknown [Streptomyces lincolnensis]	0.49

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
70	D63854	Human cytomegalovirus DNA, replication origin	1.8	1169200	DNA-DAMAGE-REPAIR/TOLE RATION PROTEIN DRT111 PRECURSOR >gi 421829 pi r S33706 DNA-damage resistance protein - Arabidopsis thaliana and DNA-damage resistance protein (DRT111) mRNA, complete cds.], gene product [Arabidopsis thaliana]	0.22
71	D26549	Bovine mRNA for adseverin, complete cds	1.8	755468	(U19879) transmembrane protein [Xenopus laevis]	0.042
72	J05211	Human desmoplakin mRNA, 3' end.	1.8	728867	ANTER- SPECIFIC PROLINE- RICH PROTEIN APG PRECURSOR >gi 99694 pir S21961 proline-rich protein APG - Arabidopsis thaliana >gi 22599 em b CAA42925	0.015

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
73	NM_004415 .1	Homo sapiens desmoplakin (DPI, DPII) (DSP) mRNA mRNA, complete cds	1.8	728867	ANTER- SPECIFIC PROLINE- RICH PROTEIN APG PRECURSOR >gi 99694 pir S21961 proline-rich protein APG - Arabidopsis thaliana >gi 22599 em b CAA42925	0.015
74	AF038604	Caenorhabditis elegans cosmid B0546	1.8	3877951	(Z81555) predicted using Genefinder	3e-008
75	AF038604	Caenorhabditis elegans cosmid B0546	1.8	3877951	(Z81555) predicted using Genefinder	2e-011
76	U23551	Prochlorothrix hollandica phosphomannom utase	1.8	2828280	(AL021687) putative protein [Arabidopsis thaliana] >gi 2832633 e mb CAA16762 (AL021711) putative protein [Arabidopsis thaliana]	2e-013
77	S60150	ORF1...ORF6 {3' terminal reigon} [chrysanthemum virus B CVB, Genomic RNA, 6 genes, 3426 nt]	1.8	1065454	(U40410) C54G7.2 gene product [Caenorhabditis elegans]	2e-019
78	AB014558	Homo sapiens mRNA for KIAA0658 protein, partial cds	1.8	3850072	(AL033385) dna-directed rna polymerase iii subunit [Schizosaccharo myces pombe]	6e-027

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
79	X17191	E.gracilis chloroplast RNA polymerase rpoB-rpoC1-rpoC2 operon	1.7	<NONE>	<NONE>	<NONE>
80	X07729	R.norvegicus gene encoding neuron-specific enolase, exons 8-12	1.7	4584544	(AL049608) extensin-like protein	8.8
81	D38178	Human gene for cytosolic phospholipase A2, exon 1	1.7	73714	infected cell protein ICP34.5 - human herpesvirus 1 (strain F) > gi 330123 (M12240) infected cell protein [Herpes simplex virus type 1]	1.1
82	U23551	Prochlorothrix hollandica phosphomannomutase	1.7	2828280	(AL021687) putative protein [Arabidopsis thaliana] > gi 2832633 emb CAA16762 (AL021711) putative protein [Arabidopsis thaliana]	2e-010
83	Y00525	Klebsiella pneumoniae nifL gene for regulatory protein	1.6	3800951	(AF100657) No definition line found [Caenorhabditis elegans]	6e-013
84	AF100170.1	Bos taurus major fibrous sheath protein precursor, mRNA, complete cds	1.5	463552	(U05877) AF-1 [Homo sapiens]	0.074
85	Y13441	Homo sapiens Rox gene, exon 2	0.74	<NONE>	<NONE>	<NONE>

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
86	L46792	Actinidia deliciosa clone AdXET-5 xyloglucan endotransglycosylase precursor (XET) mRNA, complete cds	0.73	3170252	(AF043636) circumsporozoite protein [Plasmodium chabaudi]	0.001
87	U73489	Drosophila melanogaster Nem (nem) mRNA, complete cds	0.7	3915994	HYPOTHETICAL 53.2 KD PROTEIN IN PRC-PRPA INTERGENIC REGION	3e-005
88	U95097	Xenopus laevis mitotic phosphoprotein 43 mRNA, partial cds	0.68	157272	(L11345) DNA-binding protein [Drosophila melanogaster]	8.5
89	AF082012	Caenorhabditis elegans UDP-N-acetylglucosaminase: a-3-D-mannoside b-1,2-N-acetylglucosaminyltransferase I (gly-14) mRNA, complete cds	0.67	2494313	PUTATIVE TRANSLATION INITIATION FACTOR EIF-2B SUBUNIT 1 (EIF-2B GDP-GTP EXCHANGE FACTOR) eIF-2B, subunit alpha - Methanococcus jannaschii aIF-2B, subunit delta (aIF2BD) [Methanococcus jannaschii]	8.4
90	U04354	Mus musculus ADSEVERIN mRNA, complete cds	0.67	4755188	(AC007018) unknown protein	8e-026

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
91	M68881	S.pombe cigl + gene, complete cds.	0.67	2078441	(U56964) weak similarity to S. cerevisiae intracellular protein transport protein US)1 (SP:P25386)	2e-030
92	U95097	Xenopus laevis mitotic phosphoprotein 43 mRNA, partial cds	0.66	2829685	PROTEIN-TYROSINE PHOSPHATASE X PRECURSOR (R-PTP-X) (PTP IA-2BETA) (PROTEIN TYROSINE PHOSPHATASE-NP) (PTP-NP) > gi 1515425 (U57345) protein tyrosine phosphatase-NP [Mus musculus]	6.2
93	Z15056	B.subtilis genes spoVD, murE, mraY, murD	0.66	477124	P3A2 DNA binding protein homolog EWG - fruit fly (Drosophila melanogaster)	2.1
94	M86808	Human pyruvate dehydrogenase complex (PDHA2) gene, complete cds.	0.65	< NONE >	< NONE >	< NONE >
95	J03754	Rat plasma membrane Ca2+ ATPase-isoform 2 mRNA, complete cds.	0.65	4507549	transmembrane protein with EGF-like and two follistatin-like domains 1 > gi 755466	8e-006

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
96	NM_000887.1	Homo sapiens integrin, alpha X (antigen CD11C emb Y00093 H SP15095 H.sapiens mRNA for leukocyte adhesion glycoprotein p150,95	0.64	< NONE >	< NONE >	< NONE >
97	L27080	Human melanocortin 5 receptor (MC5R) gene, complete cds.	0.64	< NONE >	< NONE >	< NONE >
98	U07890	Mus musculus C57BL/6J epidermal surface antigen (mesa) mRNA, complete cds.	0.64	< NONE >	< NONE >	< NONE >
99	AF079139	Streptomyces venezuelae pikCD operon, complete sequence	0.64	3041869	(U96109) proline-rich transcription factor ALX3 [Mus musculus]	2.8
100	M16140	Chicken ovoinhibitor gene, exon 15.	0.64	123984	ACROSIN INHIBITORS IIA AND IIB	4e-008
101	NM_000887.1	Homo sapiens integrin, alpha X (antigen CD11C emb Y00093 H SP15095 H.sapiens mRNA for leukocyte adhesion glycoprotein p150,95	0.63	< NONE >	< NONE >	< NONE >

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
102	Z17316	Kluyveromyces lactis for gene encoding phosphofructokinase beta subunit	0.63	< NONE >	< NONE >	< NONE >
103	Z25470	H.sapiens melanocortin 5 receptor gene, complete CDS	0.63	< NONE >	< NONE >	< NONE >
104	L19954	Bacillus subtilis feuA, B, and C genes, 3 ORFs, 2 complete cds's and 5'end.	0.63	< NONE >	< NONE >	< NONE >
105	U44405	Spiroplasma citri chromosome pre-inversion border, SPV1-like sequences, transposase gene, partial cds, adhesin-like protein P58 gene, complete cds.	0.63	2499642	SERINE/THREONINE-PROTEIN KINASE STE20 HOMOLOG >gi 1737181 (U73457) Cst20p [Candida albicans]	7.7

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
106	Z28264	S.cerevisiae chromosome XI reading frame ORF YKR039w	0.63	3880930	(AL021481) similar to Phosphoglucomutase and phosphomannomutase phosphoserine; cDNA EST EMBL:D36168 comes from this gene; cDNA EST EMBL:D70697 comes from this gene; cDNA EST yk373h9.5 comes from this gene; cDNA EST EMBL:T00805 ...	2e-014
107	AE001107	Archaeoglobus fulgidus section 172 of 172 of the complete genome	0.62	< NONE >	< NONE >	< NONE >
108	Z14112	B.firmus TopA gene encoding DNA topoisomerase I	0.62	310115	(L02530) Drosophila polarity gene (frizzled) homologue	0.026
109	AF118101	Toxoplasma gondii protein kinase 6 (tpk6) mRNA, complete cds	0.62	726403	(U23175) similar to anion exchange protein [Caenorhabditis elegans]	4e-018
110	M59743	Rabbit cardiac muscle Ca-2+ release channel	0.61	< NONE >	< NONE >	< NONE >
111	M12036	Human tyrosine kinase-type receptor (HER2) gene, partial cds.	0.61	61962	(X58484) gag [Simian foamy virus]	7.5

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
112	AF043195	Homo sapiens tight junction protein ZO (ZO-2) gene, alternative splice products, promoter and exon A	0.61	1572629	(U69699) unknown protein precursor [Mus musculus]	7.5
113	U18178	Human HLA class I genomic survey sequence.	0.61	1336688	(S81116) properdin [guinea pigs, spleen, Peptide, 470 aa] [Cavia]	5.7
114	U44405	Spiroplasma citri chromosome pre-inversion border, SPV1-like sequences, transposase gene, partial cds, adhesin-like protein P58 gene, complete cds.	0.61	2827531	(AL021633) hypothetical protein	3.3
115	Z33011	M.capricolum DNA for CONTIG MC008	0.61	3915729	HYPERPLASTIC DISCS PROTEIN (HYD PROTEIN) >gi 2673887 (L14644) hyperplastic discs protein	0.26
116	NM_001429.1	Homo sapiens E1A binding protein p300 mRNA, complete cds. > :: gb 162297 162297 Sequence 1 from patent US 5658784	0.61	4204294	(AC003027) lcl prt_seq No definition line found	5e-005

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
117	Z25418	C.familiaris MHC class Ib gene (DLA-79) gene, complete CDS	0.61	3877493	(Z48583) similar to ATPases associated with various cellular activities (AAA); cDNA EST EMBL:Z14623 comes from this gene; cDNA EST EMBL:D75090 comes from this gene; cDNA EST EMBL:D72255 comes from this gene; cDNA EST yk200e4.5 ...	1e-007
118	AB002150	Bacillus subtilis DNA for FeuB, FeuA, YbbB, YbbC, YbbD, YbzA, YbbE, YbbF, YbbH, YbbI, YbbJ, YbbK, YbbL, YbbM, YbbP, complete cds	0.6	<NONE>	<NONE>	<NONE>
119	Y07786	V.cholerae ORF's involved in lipopolysaccharide synthesis	0.6	<NONE>	<NONE>	<NONE>
120	Z17316	Kluyveromyces lactis for gene encoding phosphofructokinase beta subunit	0.6	<NONE>	<NONE>	<NONE>

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
121	Z71403	S.cerevisiae chromosome XIV reading frame ORF YNL127w	0.6	<NONE>	<NONE>	<NONE>
122	L34641	Homo sapiens platelet/endothelial cell adhesion molecule-1 (PECAM-1) gene, exon 10.	0.6	1147634	(U42213) micronemal TRAP-C1 protein homolog	9.6
123	AF070572	Homo sapiens clone 24778 unknown mRNA	0.6	399034	N-ACETYLMURAMOYL-L-ALANINE AMIDASE AMIB PRECURSOR > gi 628763 pir S41741 N-acetylmuramoyl-L-alanine amidase (EC 3.5.1.28) - Escherichia coli > gi 304914 (L19346) N-acetylmuramoyl-L-alanine amidase [Escherichia coli] N-acetylmuramoyl-L-alanine amidase II; a	2.5
124	X75627	C.burnetii trxB, spoIIIE and serS genes	0.6	3036833	(AJ003163) apsB [Emmericella nidulans]	0.28
125	Z99765	Flaveria pringlei gdcH gene	0.59	<NONE>	<NONE>	<NONE>

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
126	U02538	Mycoplasma hyopneumoniae J ATCC 25934 23S rRNA gene, partial sequence	0.59	< NONE >	< NONE >	< NONE >
127	Z71403	S.cerevisiae chromosome XIV reading frame ORF YNL127w	0.59	< NONE >	< NONE >	< NONE >
128	X03942	Mouse simple repetitive DNA (sqr family) transcript (clone pmlc 2) with conserved GACA/GATA repeats	0.59	< NONE >	< NONE >	< NONE >
129	U11844	Mus musculus glucose transporter (GLUT3) gene, exon 1	0.59	< NONE >	< NONE >	< NONE >
130	D63395	Homo sapiens mRNA for NOTCH4, partial cds	0.59	4433616	(AF107018) alpha-mannosidase IIx [Mus musculus]	1.8
131	Z33011	M.capricolum DNA for CONTIG MC008	0.59	3915729	HYPERPLASTIC DISCS PROTEIN (HYD PROTEIN) > gi 2673887 (L14644) hyperplastic discs protein	0.27
132	U05670	Haemophilus influenzae DL42 Lex2A and Lex2B genes, complete cds.	0.58	< NONE >	< NONE >	< NONE >

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
133	L27080	Human melanocortin 5 receptor (MC5R) gene, complete cds.	0.58	123984	ACROSIN INHIBITORS IIA AND IIB	2e-006
134	AF043195	Homo sapiens tight junction protein ZO (ZO-2) gene, alternative splice products, promoter and exon A	0.57	1572629	(U69699) unknown protein precursor [Mus musculus]	6.7
135	U57707	Bos taurus activin receptor type IIB precursor	0.57	807646	(M17294) unknown protein [Human herpesvirus 4]	0.068
136	Z17316	Kluyveromyces lactis for gene encoding phosphofructokinase beta subunit	0.56	<NONE>	<NONE>	<NONE>
137	M21535	Human erg protein (ets-related gene) mRNA, complete cds.	0.56	<NONE>	<NONE>	<NONE>
138	M64932	Candida maltosa cyclohexamide resistance protein	0.56	3219524	(AF069428) NADH dehydrogenase subunit IV [Alligator mississippiensis] >gi 3367630 emb CAA73570 (Y13113) NADH dehydrogenase subunit 4 [Alligator mississippiensis]	1.3

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
139	AE000342	Escherichia coli K-12 MG1655 section 232 of 400 of the complete genome	0.56	3874685	(Z78539) Similarity to S.pombe hypothetical protein C4G8.04 (SW:YAD4_SC HPO); cDNA EST EMBL:D27846 comes from this gene; cDNA EST EMBL:D27845 comes from this gene; cDNA EST yk202h7.3 comes from this gene; cDNA EST yk202h7.5 come...	0.088
140	Z15056	B.subtilis genes spoVD, murE, mraY, murD	0.55	477124	P3A2 DNA binding protein homolog EWG - fruit fly (Drosophila melanogaster)	3.7
141	Z58167	H.sapiens CpG island DNA genomic MseI fragment, clone 30e10, forward read cpg30e10.ft1b	0.53	<NONE>	<NONE>	<NONE>
142	M27159	Rat potassium channel-Kv2 gene, partial cds.	0.53	1850920	(U21247) Bet [Human spumaretrovirus]	0.9
143	M15555	Mouse Ig germline V-kappa-24 chain (VK24C) gene, exons 1 and 2.	0.24	<NONE>	<NONE>	<NONE>

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
144	U95097	Xenopus laevis mitotic phosphoprotein 43 mRNA, partial cds	0.24	399109	TRANSCRIPTI ON FACTOR BF-1 (BRAIN FACTOR 1) (BF1) >gi 92020 pir JH0672 brain factor 1 protein - rat >gi 203135 (M87634) BF-1 [Rattus norvegicus]	4
145	AJ002014	Crythecodium cohnii mRNA for nuclear protein JUS1	0.24	416704	BALBIANI RING PROTEIN 3 PRECURSOR balbiani ring 3 (BR3) [Chironomus tentans]	0.36
146	L35330	Rattus norvegicus glutathione S-transferase Yb3 subunit gene, complete cds.	0.23	1388158	(U58204) myomesin [Gallus gallus]	8.8
147	NM_001432 .1	Homo sapiens epiregulin (EREG) mRNA > :: dbj D30783 D3 0783 Homo sapiens mRNA for epiregulin, complete cds	0.23	2851520	TRANSFORMI NG GROWTH FACTOR ALPHA PRECURSOR (TGF-ALPHA) (EGF-LIKE TGF) (ETGF) (TGF TYPE 1) precursor - rat >gi 207282 (M31076) transforming growth factor alpha precursor [Rattus norvegicus]	2e-008

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
148	U57043	Cebus apella gamma globin (gamma1) gene, complete cds	0.22	< NONE >	< NONE >	< NONE >
149	AB023188.1	Homo sapiens mRNA for KIAA0971 protein, complete cds	0.22	< NONE >	< NONE >	< NONE >
150	M18105	Yeast (S.cerevisiae) SST2 gene encoding desensitization to alpha- factor pheromone, complete cds.	0.22	< NONE >	< NONE >	< NONE >
151	AJ001113	Homo sapiens UBE3A gene, exon 16	0.22	3122961	ENHANCER OF SPLIT GROUCHO-LIKE PROTEIN 1 > gi 2408145 (U18775) enhancer of split groucho	8.5
152	L35330	Rattus norvegicus glutathione S-transferase Yb3 subunit gene, complete cds.	0.22	1388158	(U58204) myomesin [Gallus gallus]	8.1
153	D42042	Human mRNA for KIAA0085 gene, partial cds	0.22	4827063	zinc finger protein 142 (clone pHZ-49) > gi 3123312 sp P52746 Z142_HUMAN ZINC FINGER PROTEIN 142 (KIAA0236) (HA4654) > gi 1510147 dbj BAA13242	6.1

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
154	L35330	Rattus norvegicus glutathione S-transferase Yb3 subunit gene, complete cds.	0.22	2853301	(AF007194) mucin [Homo sapiens]	1.6
155	Z11653	H.sapiens DBH gene complex repeat polymorphism DNA	0.22	3819705	(AL032824) syntaxin binding protein 1; sec1 family secretory protein [Schizosaccharomyces pombe]	1.2
156	L29063	Candida albicans fatty acid synthase alpha subunit (FAS2) gene, complete cds.	0.22	3046871	(AB003753) high sulfur protein B2E [Rattus norvegicus]	0.32
157	M64865	Horse alcohol dehydrogenase-S-isoenzyme mRNA, complete cds.	0.22	2213909	(AF004874) latent TGF-beta binding protein-2 [Mus musculus]	0.037
158	Y09472	B.taurus gene encoding preprododecapeptide	0.21	2909874	(AF047829) melatonin-related receptor [Ovis aries]	7.6
159	Y09472	B.taurus gene encoding preprododecapeptide	0.21	2909874	(AF047829) melatonin-related receptor [Ovis aries]	7.5
160	X80301	N.tabacum axi 1 gene	0.21	2832715	(AJ003066) subunit beta of the mitochondrial fatty acid beta-oxidation multienzyme complex [Bos taurus]	6

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
161	AF073485	Homo sapiens MHC class I-related protein MR1 precursor (MR1) gene, partial cds	0.21	2224559	(AB002307) KIAA0309 [Homo sapiens]	3.3
162	S78251	growth hormone receptor {alternatively spliced, exon 1B} [sheep, Merino, skeletal muscle, mRNA Partial, 438 nt]	0.21	729381	DYNAMIN-1 (DYNAMIN BREDNM19)	2
163	U16135	Synechococcus sp. Clp protease proteolytic subunit	0.21	135514	T-CELL RECEPTOR BETA CHAIN PRECURSOR precursor (ANA 11) - rabbit	0.02
164	X95601	M.hominis Imp3 and Imp4 genes	0.21	2995445	(Y10496) CDV-1 protein [Mus. musculus]	0.005
165	X95601	M.hominis Imp3 and Imp4 genes	0.21	2995447	(Y10495) CDV-1R protein [Mus. musculus]	0.005
166	AF124249.1	Homo sapiens SH2-containing protein Nsp1 mRNA, complete cds	0.21	423456	epidermal growth factor-receptor-binding protein GRB-4 - mouse (fragment)	8e-010
167	AF030282	Danio rerio homeobox protein Six7 (six7) mRNA, complete cds	0.21	3928083	(AC005770) unknown protein [Arabidopsis thaliana]	2e-014
168	X83427	O. anatinus mitochondrial DNA, complete genome	0.21	132575	RIBONUCLEASE INHIBITOR	3e-021

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
169	AJ001113	Homo sapiens UBE3A gene, exon 16	0.2	< NONE >	< NONE >	< NONE >
170	AF081533.1	Anopheles gambiae putative gram negative bacteria binding protein gene, complete cds	0.2	< NONE >	< NONE >	< NONE >
171	U70316	Dictyostelium discoideum IonA (iona) gene, partial cds	0.2	< NONE >	< NONE >	< NONE >
172	AF009341	Homo sapiens E6-AP ubiquitin-protein ligase	0.2	< NONE >	< NONE >	< NONE >
173	L35330	Rattus norvegicus glutathione S-transferase Yb3 subunit gene, complete cds.	0.2	3702275	(AC005793) KIAA0561 protein [AA 1-593] [Homo sapiens]	2.5
174	AE000573.1	Helicobacter pylori 26695 section 51 of 134 of the complete genome	0.2	3947855	(AL034381) putative Golgi membrane protein	2.5
175	X83230	G.gallus hsp90beta gene	0.2	3258596	(U95821) putative transmembrane GTPase [Drosophila melanogaster]	0.81
176	X57157	Chicken mRNA for Hsp47, heat shock protein 47	0.2	108325	insulin-like growth factor-binding protein 6	0.17

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
177	M58748	Chicken alpha-globin gene domain with structural matrix attachment sites.	0.2	1086863	(U41272) T03G11.6 gene product [Caenorhabditis elegans]	4e-005
178	AB016815	Anthocidaris crassispina mRNA for Src-type protein tyrosine kinase, complete cds	0.2	423456	epidermal growth factor-receptor-binding protein GRB-4 - mouse (fragment)	1e-012
179	AF030282	Danio rerio homeobox protein Six7 (six7) mRNA, complete cds	0.2	3928083	(AC005770) unknown protein [Arabidopsis thaliana]	3e-014
180	AL035559	Streptomyces coelicolor cosmid 9F2	0.2	2088714	(AF003139) strong similarity to NADPH oxidases; partial CDS, the gene begins in the neighboring clone	3e-022
181	S79641	SDH=succinate dehydrogenase flavoprotein subunit Mutant, 387 nt]	0.2	4755188	(AC007018) unknown protein	2e-022
182	X75383	H.sapiens mRNA for TFIIA-alpha	0.19	<NONE>	<NONE>	<NONE>
183	U53901	Hippopotamus amphibius b-casein gene, exon 7, partial cds	0.19	<NONE>	<NONE>	<NONE>
184	J05265	Mouse interferon gamma receptor mRNA, complete cds.	0.19	77356	hypothetical 70K protein - eggplant mosaic virus	0.0005

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
185	U72353	Rattus norvegicus lamin B1 mRNA, complete cds	0.19	3880857	(AL031633) cDNA EST yk404d1.5 comes from this gene; cDNA EST yk404d1.3 comes from this gene	2e-006
186	AB016815	Anthracidaris crassispina mRNA for Src-type protein tyrosine kinase, complete cds	0.19	3930217	(AF047487) Nck-2 [Homo sapiens]	2e-007
187	D10911	Mus musculus DNA for MS2 protein, complete cds	0.19	2662366	(D86332) membrane type-2 matrix metalloproteinase [Mus musculus]	5e-011
188	AB015345	Homo sapiens HRIHFB2216 mRNA, partial cds	0.075	3877417	(Z66564) similar to anion exchange protein	6.4
189	AF086410	Homo sapiens full length insert cDNA clone ZD77B03	0.075	3023371	PEROMONE B BETA 1 RECEPTOR	4.9
190	K02024	Human T-cell lymphotropic virus type II env gene encoding envelope glycoprotein, complete cds.	0.075	2791527	(AL021246) PE_PGRS [Mycobacterium tuberculosis]	0.11

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
191	M10188	X.laevis mitochondrial DNA containing the D-loop, and the 12S rRNA, apocytochrome b, Glu-tRNA, Thr-tRNA, Pro-tRNA and Phe-tRNA genes.	0.074	4753163	huntingtin DISEASE PROTEIN) (HD PROTEIN) > gi 454415 (L12392) Huntington's Disease protein [Homo sapiens]	2.8
192	X85525	G.gallus AG repeat region (GgaMU130)	0.073	984339	(U20966) Rev [Simian immunodeficiency virus]	3.6
193	AJ238394.1	Homo sapiens AML2 gene (partial)	0.07	4240219	(AB020672) KIAA0865 protein [Homo sapiens]	2
194	AF039704	Homo sapiens lysosomal pepstatin insensitive protease (CLN2) gene, complete cds	0.069	2894106	(Z78279) Collagen alpha 1 [Rattus norvegicus]	0.39
195	K02024	Human T-cell lymphotropic virus type II env gene encoding envelope glycoprotein, complete cds.	0.068	4504857	potassium intermediate/small conductance calcium-activated channel, subfamily N, member 3 > gi 3309531 (AF031815) calcium-activated potassium channel [Homo sapiens]	0.5

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
196	Z60719	H.sapiens CpG island DNA genomic MseI fragment, clone 33a11, forward read cpg33a11.ft1m	0.068	4826874	nucleoporin 214kD (CAIN) PROTEIN NUP214 (NUCLEOPORIN NUP214) (214 KD NUCLEOPORIN) transforming protein (can) - human sapiens]	0.044
197	AF053994	Lycopersicon esculentum Hcr2-0A (Hcr2-0A) gene, complete cds	0.068	2842699	PUTATIVE UBIQUITIN CARBOXYL-TERMINAL HYDROLASE C6G9.08 (UBIQUITIN THIOLESTERASE) (UBIQUITIN-SPECIFIC PROCESSING PROTEASE)	9e-009
198	AJ233650.1	Equus caballus endogenous retroviral sequence ERV-L pol gene, clone ERV-L Horse1	0.067	<NONE>	<NONE>	<NONE>
199	M10188	X.laevis mitochondrial DNA containing the D-loop, and the 12S rRNA, apocytochrome b, Glu-tRNA, Thr-tRNA, Pro-tRNA and Phe-tRNA genes.	0.067	4753163	huntingtin DISEASE PROTEIN) (HD PROTEIN) > gi 454415 (L12392) Huntington's Disease protein [Homo sapiens]	2.5

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
200	U14646	Murine hepatitis virus Y strain S glycoprotein gene, complete cds.	0.067	3880930	(AL021481) similar to Phosphoglucomutase and phosphomannomutase phosphoserine; cDNA EST EMBL:D36168 comes from this gene; cDNA EST EMBL:D70697 comes from this gene; cDNA EST yk373h9.5 comes from this gene; cDNA EST EMBL:T00805 ...	1e-019
201	X15373	Mouse cerebellum mRNA for P400 protein	0.066	164507	(M81771) immunoglobulin gamma-chain [Sus scrofa]	9.4
202	AF086410	Homo sapiens full length insert cDNA clone ZD77B03	0.066	3023371	PHEROMONE B BETA 1 RECEPTOR	4.2
203	AL034492	Streptomyces coelicolor cosmid 6C5	0.066	3800951	(AF100657) No definition line found [Caenorhabditis elegans]	3e-015
204	L13377	Staphylococcus aureus enterotoxin gene, 3' end.	0.065	<NONE>	<NONE>	<NONE>
205	U83478	Thelephoraceae sp. 'Taylor #13' ITS1, 5.8S ribosomal RNA gene, and ITS2, complete sequence	0.065	3877335	(Z92786) predicted using Genefinder	9.1

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
206	AJ002014	Crythecodium cohnii mRNA for nuclear protein JUS1	0.065	1213283	(U40576) SIM2 [Mus musculus]	0.47
207	AB016804	Aloe arborescens mRNA for NADP-malic enzyme, complete cds	0.065	2832777	(AL021086) /prediction=(method;; comes from the 5' UTR [Drosophila melanogaster])	5e-036
208	AJ002014	Crythecodium cohnii mRNA for nuclear protein JUS1	0.063	1213283	(U40576) SIM2 [Mus musculus]	0.45
209	AB023143.1	Homo sapiens mRNA for KIAA0926 protein, complete cds	0.024	132575	RIBONUCLEASE INHIBITOR	8e-026
210	U72966	Human hepatocyte nuclear factor 4-alpha gene, exon 7	0.022	< NONE >	< NONE >	< NONE >
211	X02801	Mouse gene for glial fibrillary acidic protein	0.022	2231607	(U85917) nef protein [Human immunodeficiency virus type 1]	7
212	AF017636	Mesocricetus auratus 3-ketosteroid reductase	0.022	2723362	(AF023459) lustrin A [Haliotis rufescens]	0.097
213	Z36879	F.pringlei gdcSPA gene for P-protein of the glycine cleavage system	0.008	< NONE >	< NONE >	< NONE >
214	X73150	P.sativum GapC1 gene	0.008	1572629	(U69699) unknown protein precursor [Mus musculus]	8.6

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
215	AJ239031.1	Homo sapiens LSS gene, partial, exons 22, 23 and joined CDS	0.008	4508019	zinc finger protein 231 protein [Homo sapiens]	0.01
216	U76602	Human 180 kDa bullous pemphigoid antigen 2/type XVII collagen (BPAG2/COL17A1) gene, exons 49, 50, 51 and 52	0.007	3170252	(AF043636) circumsporozoite protein [Plasmodium chabaudi]	0.0001
217	M11283	Aplysia californica FMRFamide mRNA, partial cds, clone FMRF-2.	0.007	3874685	(Z78539) Similarity to S.pombe hypothetical protein C4G8.04 (SW:YAD4_SC HPO); cDNA EST EMBL:D27846 comes from this gene; cDNA EST EMBL:D27845 comes from this gene; cDNA EST yk202h7.3 comes from this gene; cDNA EST yk202h7.5 come...	9e-013
218	J03998	P.falciparum glutamic acid-rich protein gnen, complete cds.	0.003	<NONE>	<NONE>	<NONE>
219	Z23143	M.musculus ALK-6 mRNA, complete CDS	0.002	2393890	(AF006064) protein kinase homolog [Fowlpox virus]	1e-011

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
220	AB007914	Homo sapiens mRNA for KIAA0445 protein, complete cds	0.001	2136964	cysteine-rich hair keratin associated protein - rabbit >gi 510541 emb CAA56339 (X80035) cysteine rich hair keratin associated protein	1.9
221	AB012105	Brassica rapa mRNA for SLG45, complete cds	0.0008	3687246	(AC005169) putative suppressor protein [Arabidopsis thaliana]	5.5
222	L41608	Methylobacterium extorquens (clone pDN9, HINDIIIAB) mxaS gene 3' end, mxaA, mxaC, mxaK, mxaL and mxaD genes, complete cds.	0.0008	3024235	NERVOUS-SYSTEM SPECIFIC OCTAMER-BINDING TRANSCRIPTI ON FACTOR N-OCT 3 PROTEIN)	5.1
223	AB007914	Homo sapiens mRNA for KIAA0445 protein, complete cds	0.0008	2136964	cysteine-rich hair keratin associated protein - rabbit >gi 510541 emb CAA56339 (X80035) cysteine rich hair keratin associated protein	2.5
224	AC002293	Genomic sequence from Human 9q34, complete sequence [Homo sapiens]	0.0008	2789557	(AF034316) MHC class I antigen [Triakis scyllium] scyllium]	0.0002

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
225	L16013	Rattus norvegicus Q-like gene sequence	9e-005	< NONE >	< NONE >	< NONE >
226	AF148512.1	Homo sapiens hexokinase II gene, promoter region	9e-005	< NONE >	< NONE >	< NONE >
227	U94776	Human muscle glycogen phosphorylase (PYGM) gene, exons 6 through 17	9e-005	4759138	solute carrier family 7 transporter 3 [Homo sapiens]	5.4
228	X56030	H.sapiens IAPP gene for amyloid polypeptide, exon 1	1e-005	< NONE >	< NONE >	< NONE >
229	U36515	Human CT microsatellite, clone GM5927-CT-2-3, from the tandemly repeated genes encoding U2 small nuclear RNA (RNU2 locus)	4e-007	2435616	(AF026215) No definition line found [Caenorhabditis elegans]	0.85
230	AB011119	Homo sapiens mRNA for KIAA0547 protein, complete cds	4e-007	4758508	airway trypsin-like protease [Homo sapiens]	3e-031
231	NM_000521.1	Homo sapiens hexosaminidase B (beta polypeptide) (HEXB) mRNA	5e-008	2119379	slow muscle troponin T - chicken T [Gallus gallus]	2.8
232	X13895	Human serum amyloid A (GSAAL) gene, complete cds	4e-008	699405	(U18682) novel antigen receptor [Ginglymostoma cirratum]	7.7

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
233	AB009288.1	Homo sapiens mRNA for N-copine, complete cds	4e-008	4520342	(AB008893) N-copine [Mus musculus]	3e-006
234	AB011119	Homo sapiens mRNA for KIAA0547 protein, complete cds	4e-008	4758508	airway trypsin-like protease [Homo sapiens]	1e-028
235	X13895	Human serum amyloid A (GSAA1) gene, complete cds	5e-009	699405	(U18682) novel antigen receptor [Ginglymostoma cirratum]	7.8
236	X13895	Human serum amyloid A (GSAA1) gene, complete cds	2e-009	699405	(U18682) novel antigen receptor [Ginglymostoma cirratum]	7.2
237	U64997	Bos taurus ribonuclease K6 gene, partial cds	2e-009	3914810	RIBONUCLEASE K6 PRECURSOR (RNASE K6) >gi 2745760 (AF037086) ribonuclease k6 precursor	3e-018
238	J02635	Rat liver alpha-2-macroglobulin mRNA, complete cds..	2e-009	112913	ALPHA-2-MACROGLOBULIN PRECURSOR precursor - rat >gi 202592 (J02635) prealpha-2-macroglobulin [Rattus norvegicus]	4e-019
239	Z78141	M.musculus partial cochlear mRNA (clone 29C9)	5e-010	3219569	(AL023893) /prediction =(method; ;	4e-009

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
240	AF060917	Gambusia affinis microsatellite Gafu6	2e-010	3874618	(Z48241) similar to coiled coil domains; cDNA EST yk302g12.5 comes from this gene; cDNA EST yk365d10.5 comes from this gene; cDNA EST yk461c1.5 comes from this gene [Caenorhabditis elegans] coil domains; cDNA EST yk302g12.5 comes from this gene; cDNA EST	0.096
241	U68138	Human PSD-95 mRNA, partial cds	2e-010	4521241	(AB024927) CsENDO-3 [Ciona savignyi]	2e-022
242	U88827	Aotus trivirgatus ribonuclease precursor gene, complete cds	6e-011	3914810	RIBONUCLEASE K6 PRECURSOR (RNASE K6) > gi 2745760 (AF037086) ribonuclease k6 precursor	1e-016
243	AF045573	Mus musculus FLI-LRR associated protein-1 mRNA, complete cds	2e-012	3025718	(AF045573) FLI-LRR associated protein-1 [Mus musculus]	3e-016

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
244	NM_001365 .1	Homo sapiens discs, large (Drosophila) homolog 4 (DLG4) mRNA > :: gb U83192 HS U83192 Homo sapiens post-synaptic density protein 95 (PSD95) mRNA, complete cds	2e-012	4521241	(AB024927) CsENDO-3 [Ciona savignyi]	5e-020
245	U28049	Human TBX2 (TXB2) mRNA, complete cds.	7e-013	2501115	TBX2 PROTEIN (T-BOX PROTEIN 2)	2e-011
246	M23404	Chicken erythrocyte anion transport protein (band3) mRNA, complete cds.	2e-013	726403	(U23175) similar to anion exchange protein [Caenorhabditis elegans]	1e-025
247	AF005963	Homo sapiens XY homologous region, partial sequence	1e-014	104270	Ig heavy chain - clawed frog	1.9
248	M29863	Human farnesyl pyrophosphate synthetase mRNA	9e-015	182405	(M29863) farnesyl pyrophosphate synthetase [Homo sapiens]	0.005
249	D28126	Human gene for ATP synthase alpha subunit, complete cds (exon 1 to 12)	3e-015	<NONE>	<NONE>	<NONE>

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
250	Z80150	H.sapiens CACNL1A4 gene, exons 41 and 42 > :: emb A70716.1 A70716 Sequence 37 from Patent WO9813490	3e-015	3387914	(AF070550) cote1 [Homo sapiens]	3.5
251	U28049	Human TBX2 (TXB2) mRNA, complete cds.	4e-016	2501116	TBX2 PROTEIN (T- BOX PROTEIN 2) tbx gene [Mus musculus]	6e-009
252	U31629	Mus musculus C2C12 unknown mRNA, partial cds.	1e-017	3024998	HYPOTHETIC AL HEART PROTEIN	3e-017
253	J05262	Human farnesyl pyrophosphate synthetase mRNA, complete cds.	1e-018	182405	(M29863) farnesyl pyrophosphate synthetase [Homo sapiens]	0.0001
254	D28126	Human gene for ATP synthase alpha subunit, complete cds (exon 1 to 12)	5e-019	< NONE >	< NONE >	< NONE >
255	D28126	Human gene for ATP synthase alpha subunit, complete cds (exon 1 to 12)	5e-019	3219984	HYPOTHETIC AL PROTEIN MJ1597.1 region MJ1597.1 [Methanococcus jannaschii]	5.7

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
256	NM_004587 .1	Homo sapiens ribosome binding protein 1 (dog 180kD homolog) (RRBP1) mRNA > :: gb AF006751 AF006751 Homo sapiens ES/130 mRNA, complete cds	2e-019	4759056	ribosome binding protein 1 (dog 180kD homolog) > gi 3299885 (AF006751) ES/130 [Homo sapiens]	0.004
257	U89915	Mus musculus junctional adhesion molecule (Jam) mRNA, complete cds	5e-020	3462455	(U89915) junctional adhesion molecule [Mus musculus]	2e-005
258	AF045573	Mus musculus FLI-LRR associated protein-1 mRNA, complete cds	5e-020	3025718	(AF045573) FLI-LRR associated protein-1 [Mus musculus]	9e-025
259	NM_004587 .1	Homo sapiens ribosome binding protein 1 (dog 180kD homolog) (RRBP1) mRNA > :: gb AF006751 AF006751 Homo sapiens ES/130 mRNA, complete cds	2e-020	4759056	ribosome binding protein 1 (dog 180kD homolog) > gi 3299885 (AF006751) ES/130 [Homo sapiens]	0.0008

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
260	AF051098	Mus musculus seven transmembrane domain orphan receptor mRNA, complete cds	2e-021	3858883	(U67056) myosin I heavy chain kinase [Acanthamoeba castellanii] > gi 4206769 (AF104910) myosin I heavy chain kinase [Acanthamoeba castellanii]	0.002
261	AF051098	Mus musculus seven transmembrane domain orphan receptor mRNA, complete cds	2e-021	3858883	(U67056) myosin I heavy chain kinase [Acanthamoeba castellanii] > gi 4206769 (AF104910) myosin I heavy chain kinase [Acanthamoeba castellanii]	0.001
262	M13519	Human N-acetyl-beta-glucosaminidase (HEXB) mRNA, 3' end.	2e-021	4504373	hexosaminidase B (beta polypeptide) > gi 123081 sp P07686 HEXB_HUMAN BETA-HEXOSAMINIDASE BETA CHAIN PRECURSOR beta-N-acetylhexosaminidase (EC 3.2.1.52) beta chain - human > gi 386770 (M23294) beta-hexosaminidase beta-subunit [Homo sapiens]	2e-007

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
263	Z81014	Human DNA sequence from cosmid U65A4, between markers DXS366 and DXS87 on chromosome X *	2e-022	< NONE >	< NONE >	< NONE >
264	AF147311.1	Homo sapiens full length insert cDNA clone YA82F10	2e-022	3875904	(Z70207) predicted using Genefinder; similar to collagen; cDNA EST EMBL:D65905 comes from this gene; cDNA EST EMBL:D65858 comes from this gene; cDNA EST EMBL:D69306 comes from this gene; cDNA EST EMBL:D65755 comes from this gen...	0.07
265	AF037088	Gorilla gorilla ribonuclease k6 precursor, gene, complete cds	9e-024	3914791	RIBONUCLEASE K6 PRECURSOR (RNASE K6) >gi 2745752 (AF037082) ribonuclease k6 precursor	3e-019
266	Z81014	Human DNA sequence from cosmid U65A4, between markers DXS366 and DXS87 on chromosome X	8e-024	< NONE >	< NONE >	< NONE >

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
		*				
267	AF037088	Gorilla gorilla ribonuclease k6 precursor, gene, complete cds	9e-025	3914810	RIBONUCLEASE K6 PRECURSOR (RNASE K6) >gi 2745760 (AF037086) ribonuclease k6 precursor	4e-018
268	AF147311.1	Homo sapiens full length insert cDNA clone YA82F10	1e-026	131413	PULMONARY SURFACTANT-ASSOCIATED PROTEIN A PRECURSOR (SP-A) (PSP-A) (PSAP) precursor - rabbit >gi 165706 (J03542) apoprotein of surfactant [Oryctolagus cuniculus]	0.059
269	Z46786	D.melanogaster mRNA for acetyl-CoA synthetase	1e-027	1079042	acetyl-CoA synthetase - fruit fly	4e-025
270	NM_004039.1	Homo sapiens annexin II (lipocortin II) for lipocortin II, complete cds	4e-028	450448	(M33322) calpactin I heavy chain [Mus musculus]	0.1
271	X53064	Homo sapiens SPRR2A gene encoding small proline rich protein	1e-028	134846	SMALL PROLINE-RICH PROTEIN II rich protein [Homo sapiens]	0.005

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
272	M29863	Human farnesyl pyrophosphate synthetase mRNA	1e-028	4503685	farnesyl diphosphate synthase dimethylallyltransferase, geranyltransferase) bp313 to bp1374 is almost identical to human farnesyl pyrophosphate synthetase mRNA. [Homo sapiens]	2e-008
273	Z18950	H.sapiens genes for S100E calcium binding protein, CAPL, and S100D calcium binding protein EF-Hand patent US 5789248	5e-029	2493898	DOPAMINE-BETA-MONOOXYGENASE PRECURSOR (DOPAMINE BETA-HYDROXYLASE) (DBH) 1.14.17.1) precursor - mouse > gi 260873 bb s 119249 621 aa] [Mus sp.]	1.4
274	M19481	Human follistatin gene, exon 6.	5e-030	< NONE >	< NONE >	< NONE >

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
275	AF007155	Homo sapiens clone 23763 unknown mRNA, partial cds	2e-032	4502641	chemokine (C-C) receptor 7 TYPE 7 PRECURSOR (C-C CKR-7) (CC-CKR-7) (CCR-7) (MIP-3 BETA RECEPTOR) (EBV-INDUCED G PROTEIN-COUPLED RECEPTOR 1) (EBI1) (BLR2) >gi 1082381 p ir B55735 lymphocyte-specific G-protein-coupled receptor EBI1 - human >gi 468316 (L3158	1.6
276	M99624	Human epidermal growth factor receptor-related gene, 5' end.	8e-034	294845	(L13655) membrane protein [Saccharum hybrid cultivar H65-7052]	9e-014
277	U49082	Human transporter protein (g17) mRNA, complete cds	8e-035	1840045	(U49082) transporter protein [Homo sapiens]	1e-014

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
278	D50369	Homo sapiens mRNA for low molecular mass ubiquinone-binding protein, complete cds	9e-036	3024781	UBIQUINOL-CYTOCHROME C REDUCTASE COMPLEX UBIQUINONE-BINDING PROTEIN QP-C PROTEIN) (COMPLEX III SUBUNIT VII) ubiquinone-binding protein [Homo sapiens]	0.0002
279	AF086313	Homo sapiens full length insert cDNA clone ZD52B10	9e-036	2832777	(AL021086) /prediction=(method:; comes from the 5' UTR [Drosophila melanogaster]	1e-039
280	NM_004074.1	Homo sapiens cytochrome c oxidase subunit VIII (COX8), nuclear gene encoding mitochondrial protein, mRNA > :: gb J04823 HUMCOX8A Human cytochrome c oxidase subunit VIII (COX8) mRNA, complete cds.	1e-038	2499854	PROBABLE PEPTIDASE Y4SO > gi 2182630	2
281	AB024436.1	Homo sapiens mRNA for beta-1,4-galactosyltransferase IV, complete cds	2e-041	3132900	(AF038662) beta-1,4-galactosyltransferase [Homo sapiens] beta-1,4-galactosyltransferase	4e-016

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
					galactosyltransferase IV [Homo sapiens]	
282	AF057734	Homo sapiens 17-beta-hydroxysteroid dehydrogenase IV (HSD17B4) gene, exon 16	2e-043	2842416	(AL008730) dJ487J7.1.1 (putative protein dJ487J7.1 isoform 1) [Homo sapiens]	3e-062
283	Z69650.1	Human DNA sequence from cosmid L69F7B, Huntington's Disease Region, chromosome 4p16.3 contains Huntington Disease (HD) gene	2e-044	1872200	(U22376) alternatively spliced product using exon 13A	1e-008
284	NM_003938.1	Homo sapiens adaptin, delta (ADTD) mRNA > :: gb U91930 HS U91930 Homo sapiens AP-3 complex delta subunit mRNA, complete cds	2e-044	3478639	(AC005545) delta-adaptin, partial CDS [Homo sapiens]	3e-016
285	AF026029	Homo sapiens poly(A) binding protein II (PABP2) gene, complete cds	8e-045	1916930	(U88570) CREB-binding protein homolog [Drosophila melanogaster]	7.6
286	AB006622	Homo sapiens mRNA for KIAA0284 gene, partial cds	1e-045	73404	E2 protein - human papillomavirus type 5	0.11

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
287	U90918	Human clone 23654 mRNA sequence	1e-048	3877568	(Z70208) similar to collagen	0.042
288	AB006622	Homo sapiens mRNA for KIAA0284 gene, partial cds	1e-049	73404	E2 protein - human papillomavirus type 5	0.11
289	AL049258.1	Homo sapiens mRNA; cDNA DKFZp564E173 (from clone DKFZp564E173)	1e-050	< NONE >	< NONE >	< NONE >
290	AF022367	Homo sapiens beta-1,4-galactosyltransferase mRNA, complete cds	5e-051	3132900	(AF038662) beta-1,4-galactosyltransferase [Homo sapiens] beta-1,4-galactosyltransferase IV [Homo sapiens]	6e-019
291	AF057734	Homo sapiens 17-beta-hydroxysteroid dehydrogenase IV (HSD17B4) gene, exon 16	7e-053	2842416	(AL008730) dJ487J7.1.1 (putative protein dJ487J7.1 isoform 1) [Homo sapiens]	6e-055
292	AF097709	Homo sapiens serine protease (PRSS11) mRNA, partial cds	8e-055	4506141	protease, serine, 11 (IGF binding) > gi 1513059 d bj BAA13322 (D87258) serin protease with IGF-binding motif [Homo sapiens] protease, PRSS11 [Homo sapiens]	2e-017

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
293	U31629	Mus musculus C2C12 unknown mRNA, partial cds.	9e-057	3025215	HYPOTHETICAL 81.0 KD PROTEIN C35D10.4 IN CHROMOSOME III >gi 2146877 pir S72572 probable ABC1 protein homolog - Caenorhabditis elegans protein (Swiss-Prot Acc: P27697) [Caenorhabditis elegans]	5e-033
294	AB006622	Homo sapiens mRNA for KIAA0284 gene, partial cds	8e-057	73404	E2 protein - human papillomavirus type 5	1.7
295	AF025439	Homo sapiens Opa-interacting protein OIP3 mRNA, partial cds	4e-059	<NONE>	<NONE>	<NONE>
296	M99624	Human epidermal growth factor receptor-related gene, 5' end.	1e-060	123364	SEGMENTATION PROTEIN EVEN-SKIPPED fly (Drosophila sp.) >gi 157387 (M14767) even-skipped gene [Drosophila melanogaster]	5.3
297	AF045573	Mus musculus FLI-LRR associated protein-1 mRNA, complete cds	5e-061	3025718	(AF045573) FLI-LRR associated protein-1 [Mus musculus]	7e-029
298	AB006622	Homo sapiens mRNA for KIAA0284 gene, partial cds	2e-062	2119133	ribosomal protein S17 - cat (fragment) musculus]	2e-015

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
299	M30702	Human amphiregulin (AR) gene, exon 5, clones lambda-ARH(6,12).	2e-063	4502199	amphiregulin (schwannoma-derived growth factor) >gi 113754 sp P15514 AMP R_HUMAN AMPHIREGULIN PRECURSOR (AR) (COLORECTUM CELL-DERIVED GROWTH FACTOR) (CRDGF) >gi 107391 pir A34702 amphiregulin precursor - human >gi 178890 (M30703) amphiregulin [Homo sapien	0.0002
300	L38847	Mus musculus hepatoma transmembrane kinase ligand Sequence 1 from patent US 5624899	6e-064	3861228	(AJ235272) unknown [Rickettsia prowazekii]	2.9
301	L38847	Mus musculus hepatoma transmembrane kinase ligand Sequence 1 from patent US 5624899	6e-064	3861228	(AJ235272) unknown [Rickettsia prowazekii]	2.9
302	Z78141	M.musculus partial cochlear mRNA (clone 29C9)	8e-066	1490324	(Z78141) unknown [Mus musculus]	8e-019

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
303	X12650	Mus musculus gene for beta-tropomyosin	2e-072	833602	(X54277) cardiac tropomyosin [Coturnix coturnix]	7e-022
304	M87635	Mouse beta-tropomyosin 2 mRNA, complete cds.	2e-084	1216293	(L35239) cardiac tropomyosin [Xenopus laevis]	5e-019
305	M13364	Rabbit calcium-dependent protease, small subunit mRNA, complete cds.	2e-084	115611	CALCIUM-DEPENDENT PROTEASE, SMALL NEUTRAL PROTEINASE) (CANP) >gi 108563 pir A34466 calpain (EC 3.4.22.17) II light chain - bovine 3.4.22.17) [Bos taurus]	1e-058
306	M87635	Mouse beta-tropomyosin 2 mRNA, complete cds.	3e-088	1216293	(L35239) cardiac tropomyosin [Xenopus laevis]	9e-028
307	M87635	Mouse beta-tropomyosin 2 mRNA, complete cds.	5e-092	1216293	(L35239) cardiac tropomyosin [Xenopus laevis]	2e-035
308	X85992	M.musculus mRNA for semaphorin C	8e-097	2137756	semaphorin C - mouse (fragment) musculus]	2e-048

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
309	M24103	Bovine ADP/ATP translocase T2 mRNA, complete cds.	e-103	113463	ADP,ATP CARRIER PROTEIN, LIVER ISOFORM T2 (ADP/ATP TRANSLOCASE 3) (ADENINE NUCLEOTIDE TRANSLOCATOR 3) (ANT 3) >gi 86757 pir S03894 ADP,ATP carrier protein T2 - human	2e-035
310	U48852	Cricetulus griseus HT protein mRNA, complete cds.	e-107	1216486	(U48852) HT protein [Cricetulus griseus]	3e-057
311	X76168	R.norvegicus mRNA for connexin 30.3	e-112	544118	GAP JUNCTION BETA-5 PROTEIN (CONNEXIN 30.3) (CX30.3) >gi 481577 pir S38891 connexin 30.3 - rat >gi 431204 emb CAA53762 (X76168) connexin 30.3	1e-063
312	X76168	R.norvegicus mRNA for connexin 30.3	e-115	461864	GAP JUNCTION BETA-5 PROTEIN junction protein Cx30.3 - mouse >gi 192647(M91443) connexin 30.3 [Mus musculus]	7e-064

Table 2

	Nearest Neighbor (BlastN vs. Genbank)			Nearest Neighbor (BlastX vs. Non-Redundant Proteins)		
313	AJ009634.1	Mus musculus fjx1 gene	e-137	4138203	(AJ009634) Fjx1 [Mus musculus]	5e-065
314	X76168	R.norvegicus mRNA for connexin 30.3	e-130	544118	GAP JUNCTION BETA-5 PROTEIN (CONNEXIN 30.3) (CX30.3) >gi 481577 pi r S38891 connexin 30.3 - rat >gi 431204 e mb CAA53762 (X76168) connexin 30.3	2e-074

Table 4

SEQ ID	CLUST	PairAB-text	CLONES in A	CLONES in B	RATIO PLUS	RATIO MINUS
4	819498					
8	728115	_21,22 (Normal Prostate vs. Cancerous Prostate)	6	0	5.9	
		_15,16 (Normal Colon vs. Colon Tumor)	0	7		6.62
		_16,17 (Colon Tumor vs. Colon Metastasis)	7	0	7.11	
9	372700					
		_08,09 (Lung, High Metastatic Potential vs. Lung, Low Metastatic Potential)	3	50		11.93
		_19,20 (Colon Tumor vs. Colon Tumor Metastasis)	8	0	5.98	
12	729832					
		_15,16 (Normal Colon vs. Colon Tumor)	0	11		10.41
		_16,17 (Colon Tumor vs. Colon Metastasis)	11	0	11.17	
13	505514					
		_23,24 (Normal Lung vs. Lung Tumor)	26	10	2.63	
17	549934					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	8	0	7.87	
		_16,17 (Colon Tumor vs. Colon Metastasis)	3	20		6.56
		_15,16 (Normal Colon vs. Colon Tumor)	11	3	3.88	
25	450399					

Table 4

SEQ ID	CLUST	PairAB-text	CLONES in A	CLONES in B	RATIO PLUS	RATIO MINUS
		_15,16 (Normal Colon vs. Colon Tumor)	28	68		2.3
		_15,17 (Normal Colon vs. Colon Metastasis)	28	117		3.89
26	450982					
		_16,17 (Colon Tumor vs. Colon Metastasis)	14	32		2.25
28	379302					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	8	1	7.87	
43	817503					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	18	4	4.43	
48	830085					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	0	9		9.15
52	830931					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	0	7		7.12
55	819046					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	2	13		6.61
58	728115					
		_15,16 (Normal Colon vs. Colon Tumor)	0	7		6.62
		_16,17 (Colon Tumor vs. Colon Metastasis)	7	0	7.11	
65	553242					

Table 4

SEQ ID	CLUST	PairAB-text	CLONES in A	CLONES in B	RATIO PLUS	RATIO MINUS
		_16,17 (Colon Tumor vs. Colon Metastasis)	0	6		5.91
71	820061					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	1	20		20.33
78	220584					
		_08,09 (Lung, High Metastatic Potential vs. Lung, Low Metastatic Potential)	1	12		8.59
80	549934					
		_16,17 (Colon Tumor vs. Colon Metastasis)	3	20		6.56
		_15,16 (Normal Colon vs. Colon Tumor)	11	3	3.88	
		_21,22 (Normal Prostate vs. Cancerous Prostate)	8	0	7.87	
86	819460					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	18	1	17.7	
95	551785					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	0	6		6.1
96	17092					
		_03,04 (Breast, High Metastatic Potential vs. Breast, Non-Metastatic)	0	25		25.62
99	745559					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	1	9		9.15

Table 4

SEQ ID	CLUST	PairAB-text	CLONES in A	CLONES in B	RATIO PLUS	RATIO MINUS
101	379879					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	0	9		9.15
		_08,09 (Lung, High Metastatic Potential vs. Lung, Low Metastatic Potential)	0	13		9.3
107	268290					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	33	69		2.13
108	818043					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	6	0	5.9	
114	450247					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	23	8	2.83	
115	819273					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	7	0	6.88	
116	587779					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	6	0	5.9	
118	615617					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	0	7		7.12
121	818682					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	11	2	5.41	

Table 4

SEQ ID	CLUST	PairAB-text	CLONES in A	CLONES in B	RATIO PLUS	RATIO MINUS
123	484413					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	7	0	6.88	
124	819273					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	7	0	6.88	
127	818682					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	11	2	5.41	
131	819273					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	7	0	6.88	
147	820061					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	1	20		20.33
153	375958					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	2	11		5.59
		_08,09 (Lung, High Metastatic Potential vs. Lung, Low Metastatic Potential)	0	9		6.44
155	831049					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	0	11		11.18
157	553200					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	0	6		6.1

Table 4

SEQ ID	CLUST	PairAB-text	CLONES in A	CLONES in B	RATIO PLUS	RATIO MINUS
158	139677					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	6	0	5.9	
159	139677					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	6	0	5.9	
163	375958					
		_08,09 (Lung, High Metastatic Potential vs. Lung, Low Metastatic Potential)	0	9		6.44
		_21,22 (Normal Prostate vs. Cancerous Prostate)	2	11		5.59
168	831812					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	0	7		7.12
176	193373					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	6	0	5.9	
177	400619					
		_08,09 (Lung, High Metastatic Potential vs. Lung, Low Metastatic Potential)	6	0	8.38	
178	831149					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	0	7		7.12
180	817503					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	18	4	4.43	

Table 4

SEQ ID	CLUST	PairAB-text	CLONES in A	CLONES in B	RATIO PLUS	RATIO MINUS
187	648679					
		_23,24 (Normal Lung vs. Lung Tumor)	11	1	11.11	
		_16,17 (Colon Tumor vs. Colon Metastasis)	79	0	80.23	
		_15,17 (Normal Colon vs. Colon Metastasis)	7	0	7.51	
		_15,16 (Normal Colon vs. Colon Tumor)	7	79		10.68
190	373928					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	7	0	6.88	
195	373928					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	7	0	6.88	
198	372700					
		_19,20 (Colon Tumor vs. Colon Tumor Metastasis)	8	0	5.98	
		_08,09 (Lung, High Metastatic Potential vs. Lung, Low Metastatic Potential)	3	50		11.93
204	379105					
		_15,16 (Normal Colon vs. Colon Tumor)	0	8		7.57
205	831188					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	0	8		8.13
209	831812					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	0	7		7.12

Table 4

SEQ ID	CLUST	PairAB-text	CLONES in A	CLONES in B	RATIO PLUS	RATIO MINUS
213	831026					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	0	10		10.17
215	380207					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	0	6		6.1
		_08,09 (Lung, High Metastatic Potential vs. Lung, Low Metastatic Potential)	0	8		5.72
216	819460					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	18	1	17.7	
224	819201					
		_21,22 (Normal Prostate vs. Cancerous Prostate)	6	0	5.9	
225	374826					
		_15,17 (Normal Colon vs. Colon Metastasis)	5	20		3.73
		_08,09 (Lung, High Metastatic Potential vs. Lung, Low Metastatic Potential)	38	132		2.49
		_15,16 (Normal Colon vs. Colon Tumor)	5	18		3.41
231	553242					
		_16,17 (Colon Tumor vs. Colon Metastasis)	0	6		5.91
246	220584					
		_08,09 (Lung, High Metastatic Potential vs. Lung, Low Metastatic Potential)	1	12		8.59
248	819498					

Table 4

SEQ ID	CLUST	PairAB-text	CLONES in A	CLONES in B	RATIO PLUS	RATIO MINUS
		21,22 (Normal Prostate vs. Cancerous Prostate)	6	0	5.9	
253	819498					
		21,22 (Normal Prostate vs. Cancerous Prostate)	6	0	5.9	
256	831160					
		21,22 (Normal Prostate vs. Cancerous Prostate)	0	12		12.2
259	831160					
		21,22 (Normal Prostate vs. Cancerous Prostate)	0	12		12.2
262	373298					
		15,17 (Normal Colon vs. Colon Metastasis)	126	42	3.22	
		15,16 (Normal Colon vs. Colon Tumor)	126	59	2.26	
270	450262					
		21,22 (Normal Prostate vs. Cancerous Prostate)	0	8		8.13
271	484703					
		21,22 (Normal Prostate vs. Cancerous Prostate)	28	0	27.54	
272	819498					
		21,22 (Normal Prostate vs. Cancerous Prostate)	6	0	5.9	

Table 4

SEQ ID	CLUST	PairAB-text	CLONES in A	CLONES in B	RATIO PLUS	RATIO MINUS
273	406043					
		21,22 (Normal Prostate vs. Cancerous Prostate)	0	6		6.1
274	817500					
		21,22 (Normal Prostate vs. Cancerous Prostate)	2	18		9.15
275	818180					
		21,22 (Normal Prostate vs. Cancerous Prostate)	2	10		5.08
280	429009					
		21,22 (Normal Prostate vs. Cancerous Prostate)	8	1	7.87	
284	383021					
		21,22 (Normal Prostate vs. Cancerous Prostate)	3	12		4.07
289	831580					
		21,22 (Normal Prostate vs. Cancerous Prostate)	0	6		6.1
311	763446					
		21,22 (Normal Prostate vs. Cancerous Prostate)	11	1	10.82	
312	763446					
		21,22 (Normal Prostate vs. Cancerous Prostate)	11	1	10.82	
314	763446					
		21,22 (Normal Prostate vs. Cancerous Prostate)	11	1	10.82	
315	10154					
		_3,4 (Breast,High Metastatic Potential vs. Breast, Low Metastatic)	3	317		108.1

Table 7

Library No.	Clones		
es75	M00063947D:D01 M00063158A:A01 M00063517A:A04 M00063520D:E11 M00063638C:G12 M00063642B:A08 M00063686B:E07 M00063689D:E12 M00063781B:B10 M00063826A:D03	es79	M00064003B:C10 M00064302A:D10 M00064309C:H09 M00064310D:F03 M00064322C:A10 M00064359B:H12 M00064390A:C05 M00064404A:B05 M00064404C:G05 M00064404D:A06
es76	M00063838B:G08 M00063838B:G08 M00063841A:B09 M00063886A:B06 M00063910D:A12 M00063912A:D06 M00063920D:H05 M00063928A:G09 M00063934B:E04 M00063945A:C03	es80	M00064429D:B07 M00064446A:D11 M00064457D:C09 M00064476D:C04 M00064506A:C07 M00064514A:G10 M00064520A:F08 M00064579D:E11 M00064620C:D01 M00064624D:C09
es77	M00064032D:G04 M00064046A:G02 M00064053C:G04 M00064053D:F02 M00064082A:A08 M00064089B:F09 M00064132B:B07 M00064138A:F11 M00064161B:G04 M00064175B:B09	es81	M00064633C:A03 M00064637B:F03 M00064690A:C04 M00064690A:C04 M00064714A:G03 M00064723D:H11 GKC10154-1 GKC10154-3
es78	M00064178C:C04 M00064179A:C04 M00064200D:E08 M00064248A:E02 M00064270B:B03 M00064271B:D03 M00063580C:A06 M00063594B:H07 M00064002C:F06 M00064002C:H09		

es82

M00063151A:G06	M00063852D:F07
M00063151D:B10	M00063888D:D05
M00063152C:B07	M00063888D:F02
M00063156D:H10	M00063890A:F11
M00063158A:E11	M00063890A:H04
M00063158A:E11	M00063891A:F11
M00063452A:F08	M00063892B:G02
M00063453B:F08	M00063898A:A10
M00063462D:D07	M00063915C:E01
M00063463D:B05	M00063919C:E07
M00063466C:C11	M00063920D:H02
M00063467D:H07	M00063922B:A12
M00063478C:D01	M00063925B:F04
M00063482A:A08	M00063926A:H04
M00063482A:F07	M00063931B:E10
M00063485A:E05	M00063931B:F07
M00063487C:C02	M00063932D:G08
M00063514C:D03	M00063934C:C10
M00063514C:E08	M00063938B:H07
M00063515B:F06	M00063939C:D06
M00063515B:H02	M00063939C:H01
M00063518D:A01	M00063940D:F09
M00063520D:D08	M00063940D:F09
M00063604A:B11	M00063941B:C12
M00063606C:B04	M00063943B:G12
M00063610D:C11	M00063949D:A05
M00063613D:C11	M00064021D:H01
M00063617D:F09	M00064025D:E07
M00063627C:F06	M00064025D:H12
M00063636A:E01	M00064033C:C11
M00063681B:C02	M00064033D:B01
M00063682A:C04	M00063843B:D07
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M00063801B:D04	M00063841A:E08
M00063804C:A11	
M00063805D:E05	
M00063807A:D12	
M00063810C:E03	

es83

M00064043D:C09	M00063577C:C02
M00064048C:G12	M00063578B:E02
M00064053B:D09	M00063578C:A06
M00064057C:H10	M00063580D:B06
M00064059A:C11	M00063593A:D03
M00064060B:D03	M00063600C:C09
M00064079C:A10	M00063955C:F07
M00064082D:D10	M00063955D:F05
M00064083D:E05	M00063956A:F05
M00064086C:E01	M00063957A:E02
M00064090C:A02	M00063957A:E02
M00064090D:D09	M00063967C:A12
M00064105B:A03	M00063967D:G02
M00064106C:G03	M00063968D:G08
M00064113B:C04	M00063972C:E10
M00064115B:E12	M00063978B:B06
M00064119B:H10	M00063981D:A06
M00064119C:D12	M00063990A:D05
M00064122C:B06	M00063990A:D05
M00064126C:C02	M00063997C:B12
M00064126C:F12	M00063998C:E09
M00064136C:D12	M00064000B:C03
M00064144D:A07	M00064001A:B03
M00064151B:C07	M00064005D:A08
M00064159A:H03	M00064008A:B01
M00064165A:B12	M00064009A:C01
M00064171D:E05	M00064014D:H05
M00064171D:E05	M00064018C:E07
M00064172C:A02	M00064293D:B12
M00064173B:E01	M00064294D:F01
M00064176D:H10	M00063557D:C07
M00064178B:A05	M00063559D:G03
M00064178B:A05	M00063571B:G03
M00064180A:G03	M00063575B:G02
M00064186C:B03	M00063555B:D01
M00064188B:G08	M00063533A:C12
M00064194C:D02	M00063534C:A02
M00064212D:E04	M00063538D:B01
M00064260C:E05	M00063539C:C11
M00064268D:G03	
M00064272C:G01	
M00063163A:G04	
M00063165A:C09	

es84

M00064307B:G02	M00064564A:C02
M00064307C:G03	M00064568A:H06
M00064310C:A10	M00064569B:A09
M00064328B:H04	M00064569B:A09
M00064328B:H09	M00064571C:C04
M00064337D:F01	M00064577C:B120
M00064341A:C02	M00064579A:C06
M00064345A:A03	M00064593A:A05
M00064346C:B09	M00064593D:C01
M00064349D:H01	M00064601C:G07
M00064352C:H01	M00064601D:B05
M00064354A:A10	M00064605C:G05
M00064358A:G03	M00064610D:H01
M00064358C:D09	M00064620D:G05
M00064375B:G07	M00064624C:B03
M00064376A:A05	M00064631A:C07
M00064385D:C11	M00064631A:C07
M00064386B:C02	M00064631C:H11
M00064386B:C02	M00064636B:A04
M00064393B:H04	M00064649A:E04
M00064399A:E01	M00064650B:B07
M00064405B:C04	M00064652B:D09
M00064406B:H06	M00064675C:E09
M00064414D:D06	M00064678D:F05
M00064415B:G03	M00064693D:F08
M00064424B:C12	M00064723C:H04
M00064428B:A12	M00064723D:H03
M00064447B:A07	M00064723D:H03
M00064447B:C06	M00003773D:H02
M00064450C:E07	M00021929A:D03
M00064452D:E11	M00043134A:A05
M00064454A:H10	M00064534D:F06
M00064454C:B06	M00064550A:A07
M00064460C:B01	M00064554D:A03
M00064467B:D06	M00064526D:F05
M00064481C:F03	M00064527A:H07
M00064508A:B09	M00064530B:H02
M00064514D:F11	M00064532D:G06
M00064517B:F04	M00064520A:E04
M00064517B:F10	M00064520A:E04
M00064517C:F11	M00064524A:A09

Table 8 Patient ID	Path Report ID	Anatomical Loc	Primary Tumor Size	Primary Tumor Grade	Histopath Grade	Local Invasion	Lymphnode Met	Incidence Lymphnode Met	Regional Lymphnode Grade	Distant Met & Loc	Descrip Distant Met	Dist Met Grade	Comment
15	21	Ascending colon,	4.0	T3	G2	extending into subserosal adipose tissue	positive	3/8	N1	negative		MX	invasive adenocarcin oma, moderately differentiate d; focal perineural invasion is seen
52	71	Ascending colon	9.0	T3	G3	Invasion through muscularis propria, subserosal involvement; ileocec. valve involvement	negative	0/12	N0	negative		M0	Hyperplastic polyp in appendix.
121	140	Sigmoid	6	T4	G2	Invasion of muscularis propria into serosa, involving submucosa of urinary bladder	negative	0/34	N0	negative		M0	Perineural invasion; donut anastomosis negative. One tubulovillou s and one tubular adenoma with no high

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													grade dysplasia.
125	144	Cecum	6	T3	G2	Invasion through the muscularis propria into serosal adipose tissue. Ileocecal junction.	negative	0/19	N0	negative		M0	patient history of metastatic melanoma
128	147	Transverse colon	5.0	T3	G2	Invasion of muscularis propria into percolonic fat	positive	1/5	N1	negative		M0	
130	149	Splenic flexure	5.5	T3		through wall and into surrounding adipose tissue	positive	10/24	N2	negative		M1	

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133	152	Rectum	5.0	T3	G2	Invasion through muscularis propria into non- peritonealized pericolonic tissue; gross configuration is annular.	negative	0/9	N0	negative		M0	Small separate tubular adenoma (0.4 cm)
141	160	Cecum	5.5	T3	G2	Invasion of muscularis propria into pericolonic adipose tissue, but not through serosa. Arising from tubular adenoma.	positive	7/21	N2	positive (Liver)	adenoca rcinoma consista nt with primary	M1	Perineural invasion identified adjacent to metastatic adenocarcin oma.

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156	175	Hepatic flexure	3.8	T3	G2	Invasion through mucularis propria into subserosa/peric olic adipose, no serosal involvement. Gross configuration annular.	positive	2/13	N1	negative		M0	Separate tubulovillou s and tubular adenomas
228	247	Rectum	5.8	T3	G2 to G3	Invasion through mucularis propria to involve subserosal, perirectoal adipose, and serosa	positive	1/8	N1	negative		MX	Hyperplastic polyps

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264	283	Ascending colon	5.5	T3	G2	Invasion through muscularis propria into subserosal adipose tissue.	negative	0/10	N0	negative		M0	Tubulovillo us adenoma with high grade dysplasia
266	285	Transverse colon	9	T3	G2	Invades through muscularis propria to involve pericolonic adipose, extends to serosa.	negative	0/15	N1	positive (Mesenteric deposit)	0.4 cm, may represent lymph node complet ely replaced by tumor	MX	
268	287	Cecum	6.5	T2	G2	Invades full thickness of muscularis propria, but mesenteric adipose free of malignancy	negative	0/12	N0	negative		M0	

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278	297	Rectum	4	T3	G2	Invasion into perirectal adipose tissue.	positive	7/10	N2	negative		M0	Descending colon polyps, no HGD or carcinoma identified..
295	314	Ascending colon	5.0	T3	G2	Invasion through muscularis propria into pericolic adipose tissue.	negative	0/12	N0	negative		M0	Melanosis coli and diverticular disease.
339	358	Rectosigmoid	6	T3	G2	Extends into perirectal fat but does not reach serosa	negative	0/6	N0	negative		M0	hyperplastic polyp identified
341	360	Ascending colon	2 cm invasive	T3	G2	Invasion through muscularis propria to involve pericolonic fat. Arising from villous adenoma.	negative	0/4	N0	negative		MX	

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356	375	Sigmoid	6.5	T3	G2	Through colon wall into subserosal adipose tissue. No serosal spread seen.	negative	0/4	N0	negative		M0	
360	412	Ascending colon	4.3	T3	G2	Invasion thru muscularis propria to pericolonic fat	positive	1/5	N1	negative		M0	Two mucosal polyps
392	444	Ascending colon	2	T3	G2	Invasion through muscularis propria into subserosal adipose tissue, not serosa.	positive	1/6	N1	positive (Liver)	Macrovesicular and microvesicular steatosis	M1	Tumor arising at prior ileocolic surgical anastomosis.

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393	445	Cecum	6.0	T3	G2	Cecum, invades through muscularis propria to involve subserosal adipose tissue but not serosa.	negative	0/21	N0	negative		M0	redagnosis of oophorectomy path to metastatic colon cancer.
413	465	Ascending colon	4.8	T3	G2	Invasive through muscularis to involve periserosal fat; abutting ileocecal junction.	negative	0/7	N0	positive (Liver)	adenocarcinoma in multiple slides	M1	

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505	383		7.5 cm max dim	T3	G2	Invasion through muscularis propria involving pericolonic adipose, serosal surface uninvolved		2/17	N1	positive (Liver)	moderately differentiated adenocarcinoma, consistent with primary	M1	Anatomical location of primary not notated in report. Evidence of chronic colitis.
517	395	Sigmoid	3	T3	G2	penetrates muscularis propria, involves pericolonic fat.	positive	6/6	N2	negative		M0	No mention of distant met in report

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534	553	Ascending colon	12	T3	G3	Invasion through the muscularis propria involving pericolonic fat. Serosa free of tumor.	negative	0/8	N0	negative		M0	Omentum with fibrosis and fat necrosis. Small bowel with acute and chronic serositis, focal abscess and adhesions.
546	565	Ascending colon	5.5	T3	G2	Invasion through muscularis propria extensively through submucosal and extending to serosa.	positive	6/12	N2	positive (Liver)	metastatic adenocarcinoma	M1	

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						Invasion through the bowel wall, into suberosal adipose. Serosal surface free of tumor.							Appendix dilated and fibrotic, but not involved by tumor
577	596	Cecum	11.5	T3	G2		negative	0/58	N0	negative		M0	tubular adenoma and hyperplastic polyps present, moderately differentiated adenoma with mucinous differentiation (% not stated)
695	714	Cecum	14	T3	G2		negative	0/22	N0	negative		MX	invasive poorly differentiated adenosquamous
784	803	Ascending colon	3.5	T3	G3	through muscularis propria into pericolic soft tissues	positive	5/17	N2	positive (Liver)		M1	

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													carcinoma
786	805	Descending colon	9.5	T3	G2	through muscularis propria into pericolic fat, but not at serosal surface	negative	0/12	N0	positive (Liver)		M1	moderately differentiated invasive adenocarcinoma
791	810	Ascending colon	5.8	T3	G3	through the muscularis propria into pericolic fat	positive	13/25	N2	positive (Liver)		M1	poorly differentiated invasive colonic adenocarcinoma

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						into muscularis propria							well- to moderately- differentiated adenocarcin oma; this patient has tumors of the ascending colon and the sigmoid colon
888	908	Ascending colon	2.0	T2	G1		positive	3/21	N0	positive (Liver)		M1	
889	909	Cecum	4.8	T3	G2	through muscularis propria int subserosal tissue	positive	1/4	N1	positive (Liver)		M1	moderately differentiated adenocarcin oma